CYSTATIN-C AND CREATININE BASED FORMULAE FOR ESTIMATION OF GLOMERULAR FILTRATION RATE IN RENAL ALLOGRAFT RECIPIENTS

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BONAFIDE CERTIFICATE

This is to certify that the work presented in this dissertation titled "**CYSTATIN-C AND CREATININE BASED FORMULAE FOR ESTIMATION OF GLOMERULAR FILTRATION RATE IN RENAL ALLOGRAFT RECIPIENTS**" done towards fulfillment of the requirements of the **Tamil Nadu Dr. M.G.R. Medical University, Chennai for the D.M. (Branch–III) (Nephrology)** exams to be conducted in July 2008, is a bonafide work of the candidate **Dr.Basu.G**., Senior Post graduate student in the Department of Nephrology, Christian Medical College, Vellore under my guidance and supervision. This dissertation has not been submitted, fully or in part to any other board or University.

Guide & Head of Dept.:

Dr. George T. John M.D., D.M.,MNAMS, FRACP., FRCP,
Professor and Head,
Department of Nephrology,
Christian Medical College,
Vellore – 632004

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Contents

ABBREVIATIONS

GFR – Glomerular Filtration Rate

S.Cr – Serum Creatinine

CysC – Cystatin C

eGFR – Estimated glomerular filtration rate

mGFR – Measured glomerular filtration rate

BSA – body surface area

IBW – ideal body weight

LBW – lean body weight

CGGFR – Cockcroft Gault GFR

CGLW - Cockcroft Gault GFR with lean body weight

CGIW - Cockcroft Gault GFR with ideal body weight

MDRD – Modification of diet in renal disease

NKVL – Nankivell GFR

MCQ – Mayo Clinic Quadratic GFR

LEB – Le Bricon Cystatin C GFR

LSN – Larsson Cystatin C GFR

GA – Grubb Age adjusted Cystatin C GFR

GAS - Grubb Age and Sex adjusted Cystatin C GFR

HK – Hoeck Cystatin C GFR

MCTX – Mayo Clinic Cystatin C Transplant GFR

MAC – Macissac Cystatin C GFR

ZCOM – Zuo Combined GFR

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ABSTRACT:

AIM: To assess the performance characteristics of the various eGFR equations based on serum creatinine and serum Cystatin C in predicting the measured GFR in renal allograft recipients of Indian Subcontinent

PATIENTS AND METHODS: Renal allograft recipients were studied at 6 months post transplant. GFR was measured by 99mTcDTPA scintigraphy (mGFR). Serum CystatinC & creatinine were measured. GFR was estimated (eGFR) by various S.Cr based and CysC based equations and compared with mGFR using correlation, bias, precision, accuracy and kappa statistics for agreement.

RESULTS: 134 renal allograft recipients (M:F=101:33; age 34.0±11.5years) were studied. Mean mGFR was 52.1±22.6ml/min/1.73Sq.m. Overall, the S.Cr-eGFRs overestimated GFR [+15to20±25ml/min/1.73Sq.m.] and had poor correlation (ICC=0.0-0.4), poor accuracy (@30%-35%; @50%-55%) and poor agreement (K= 0.05-0.20; 50-60% misclassified) with mGFR. Among them, CG-LWGFR had marginal correlation (ICC=0.25-0.35), least bias (+4.0±22.4ml/min/1.73Sq.m) and most accuracy (@30%-47.8%; @50%-72.1%). All CysC-eGFRs had better correlation (ICC=0.45-0.50), lower bias (-10to+1±20ml/min/1.73Sq.m), higher accuracy (@30%-60%; @50%-80%) and better agreement (K=0.25-0.45; 35-40% misclassified) with the mGFR. The Larsson, Macissac and MayoClinic CysC GFRs had the best performance characteristics.

CONCLUSION: In renal allograft recipients, S.Cr-eGFR estimates poorly correlate, widely differ and rarely agree with the mGFR, compared to CysC-eGFR. The CysC-eGFRs by Larsson, Macissac and MayoClinic provide the best GFR estimates. CysC is a better predictor of GFR than S.Cr at 6 months post transplantation.

INTRODUCTION

Research in renal transplantation has so far considered one-year acute rejection episodes, one-year graft and patient survival as short-term primary outcomes in evaluating therapeutic strategies. With recent advances in immunosuppression and care of a transplant recipient, the short-term renal transplant outcomes have been remarkably excellent. This makes evaluation of newer therapeutic strategies with such outcomes difficult. Meanwhile long-term results do not correlate well with these traditional short-term end-points. Glomerular filtration rate is used as an indicator of renal function worldwide. Renal function in the short term has well been related to long term outcomes in renal transplantation. This calls for use of Glomerular filtration rate as a significant short term transplant outcome for further studies evaluating efficacy of newer therapeutic strategies and to identify prognostic indicators. Renal graft function assessment is now a primary criterion for evaluation of newer therapeutic interventions in transplantation. Assessment of renal function in a transplant recipient has always been a challenge. Most of currently existing methods of GFR measurement and estimation are not well validated in transplant recipients. Inulin Clearance is considered as goal standard in measurement of renal graft function. However, other traditionally accepted reference methods for direct measurement of GFR include use of exogenous markers like radiolabeled isotopes (^{51}Cr EDTA, ^{99m}Tc-DTPA or ^{125}I Iothalamate) and non-radioactive contrast agents (Iothalamate or Iohexol). Measurement of GFR in Indian population has been limited to the reference methods only as Inulin is currently not available in this country. In view of the cumbersome nature of these tests, several mathematical formulae based on endogenous markers of GFR such as S. Creatinine and Cystatin C have been developed

for prediction of the GFR based on the abovementioned reference methods. However, these equations have been shown to have variable performance in various studies in transplant population. Among the several equations based on serum creatinine, the MDRD (abbreviated) was found to have least bias and best accuracy. Cystatin C estimates of GFR are studied recently and have been found to be precise and accurate compared to creatinine based GFR estimates. However, GFR estimates have not been well validated in the transplant population in India. Hence, it is important to study the various formulae estimates with available reference method for estimation of GFR.

OBJECTIVES

To study the agreement between GFR estimated by various prediction formulae based on Serum Creatinine and Cystatin C with the GFR measured by ^{99m}Tc DTPA renal scintigraphy among renal allograft recipients.

To determine which estimated GFR prediction formula predicts GFR measured by ^{99m}Tc DTPA renal scintigraphy with precision and accuracy in renal allograft recipients.

REVIEW OF LITERATURE

1.0 GLOMERULAR FILTRATION RATE (GFR):

The Glomerular Filtration Rate (GFR) is the volume of isosmotic plasma ultrafiltrate filtered through the glomeruli in all the nephrons of the functioning kidney(s) or renal allograft. It is equal to the sum of the filtration rates in each of the functioning nephrons. Therefore, GFR is considered an index of the function of the kidney. It is a function of the renal blood flow and the filtration coefficient of the filtration barrier (glomerular basement membrane with the endothelial fenestrae and podocyte-slit diaphragms). The number of functioning nephrons affects GFR to a certain extent. A reduction in GFR implies either progression of the primary disease or the development of a superimposed secondary pathology. An increase in GFR, on the other hand, is indicative of improvement in renal function, whereas a stable GFR implies stable disease. However, there is no absolute correlation between loss of renal mass and loss of renal function. The kidney adapts to loss in nephrons by compensatory hyperfiltration and increased solute and water reabsorption in the remaining normal nephrons (single nephron GFR). Normal GFR in an adult with normal functioning kidneys is related to age, sex, and body size. Usually a value of 130 ml/min/1.73Sq.m is considered as normal in young men and 120 ml/min/1.73 Sq.m in young women. There is an age dependent reduction in GFR. After age 20 to 30 years, GFR decreases by approximately 1.0 ml/min/1.73Sq.m /year.

2.0 ASSESSMENT OF GFR:

GFR estimation is important for detection, appropriate classification, management, follow up and prognostication of renal disease. The Kidney/Dialysis Outcome Quality Initiative (K/DOQI) guidelines[1] have published recommendations on classifying patients by chronic kidney disease stage based on the estimation of the GFR. There are several ways to measure or estimate GFR. GFR can be measured directly or estimated using indirect markers.

2.1 DIRECT METHODS:

The ideal method of directly measuring GFR would be to measure the urinary or plasma clearance of an ideal filtration marker. An ideal filtration marker should be

- Physiologically inert,
- With a stable concentration in the plasma,
- Not protein bound in blood,
- Freely filtered at the glomerulus,
- Not secreted,
- Not reabsorbed,
- Not synthesized and
- Not metabolized by the kidney.

The principle behind the direct methods is that the amount of the substance filtered at the glomerulus is equal to the amount excreted in the urine. The excreted amount can be measured. The abovementioned properties are important for exact measurement of GFR using a filtration marker. Any substance used for measuring GFR should fulfill these

attributes. Inulin is the closest to the ideal filtration marker. Hence, inulin clearance is the gold standard for determination of GFR. However, Inulin is not freely available and cannot be used frequently. Accurate determination of the GFR is also possible using the clearance of a radiolabelled compound such as radiolabelled Iothalamate (^{131}I), DTPA (^{99m}Tc), or ethylenediaminetetraacetic acid (^{51}Cr EDTA). Radio contrast agents like Iohexol and non-radiolabelled Iothalamate are also useful for this purpose[2]. The direct method is considered as complex, expensive and difficult to perform technique in routine clinical practice. Using the Direct methods, measurement error is reported to be around 5 - 20 % (This can mean 5-20% variation within a single clearance procedure / between clearance procedures on different days). This variation is greater in the higher ranges of GFR on the absolute scale. Most of these methods are impractical and expensive for use on a regular basis.

2.2 INDIRECT METHODS:

As an alternative, a number of easy-to-use mathematical equations, incorporating different anthropometric variables and biological parameters, have been developed to predict ('estimated GFR'), rather than to directly measure GFR ('measured GFR'). The most common methods utilized to estimate the GFR are the creatinine clearance, and estimation equations based upon the plasma creatinine concentration, namely the Cockcroft-Gault (CG) equation[3] and Modification of Diet in Renal Disease (MDRD) equations[4]. The abbreviated MDRD equation (also known as the four-variable MDRD equation) is a simplified version, and is being increasingly utilized[5]. NKF – K-DOQI guidelines recommend that GFR should be estimated from prediction equations that take

into account the serum creatinine concentration along with age, gender, race, and body size It is important to be familiar with the GFR rather than the serum creatinine value while managing patients with renal disease. In adults, the MDRD Study equation and Cockcroft-Gault equations are recommended. In children, the Schwartz formula[6] and the Counahan-Barratt equations[7] are most popular.

2.3 STANDARDIZATION TO BODY SURFACE AREA:

The basic function of the kidneys is to clear the waste produced by the body. Hence, it is necessary to index GFR for a measure of body size in order to compare values of GFR between individuals. In the nineteenth century, it became widely accepted that the metabolic rate of an animal is closely related to body surface area (BSA) regardless of species[8]. As a result, it was routine practice to index physiological variables to BSA. BSA is calculated by a 'height–weight' formula derived by Du Bois brothers from sophisticated calculations based on measurement of body surface area from 11 individuals with widely varying body habitus (including one child)[9] . McIntosh et al. proposed indexing kidney function to BSA in one of the early papers describing the concept of renal clearance[10]. They proposed 1.73 Sq.m as the index value as this was found to be the average calculated BSA of 25-year-old Americans of that time. Though BSA calculated from height–weight formulae of adults in most countries are larger or smaller than this number, it is important to note that this is an arbitrarily chosen value and that the index value should remain constant to facilitate studies that use historical and regional comparisons. However, in clinical evaluation and management of a patient, it is important to use the estimated GFR (eGFR) which is estimated for the individual

patient's BSA rather than the standardized value extrapolated for a 1.73Sq.m BSA. A physiologically appropriate index of standardization is probably the Extracellular Fluid Volume (ECFV)[11]. Nevertheless, for most situations where a measure of kidney function is required, it is practical to accept the physiological flaws and sacrifice of accuracy for simplicity inherent in indexing GFR for BSA.

2.4 GFR ASSESSMENT IN RENAL TRANSPLANTATION:

In renal transplantation estimation of GFR using serum creatinine has a great disadvantage, since massive reductions in GFR at the higher ranges manifest as very modest increments in serum creatinine. It is clear that larger numbers estimating a physiologically relevant parameter (eGFR) is definitely more useful and easy to understand than small numbers that are inversely and nonlinearly related to the relevant physiological parameter (serum creatinine in mg/dl or μmol/L). Following up eGFR rather than just serum creatinine values helps to detect graft dysfunction and react to the same at an earlier stage.

Over the past two decades, there have been substantial improvements in short-term kidney transplant outcome primarily due to advances in immunosuppression, diagnostics and therapeutics, understanding of the transplant immunobiology and in overall care of the recipient. As the short term end-points like the 1 year acute rejection rates or 1 year graft and patient survival are already excellent, our ability to assess the efficacy of newer therapeutic strategies using these conventional short-term end points is limited, logistically challenging and clinically questionable. Meanwhile, these traditional end-points have failed to predict long-term survival, which is currently a major challenge in

renal transplantation. Short-term end-points that correlate with long-term graft outcome such as post-transplant graft function seems to be an attractive alternative end point for clinical trials[12] , even though its use as a true surrogate marker for allograft loss has been disputed[13,14] , . Renal allograft function is the primary criterion for the evaluation of novel immunosuppressive strategies in several recent studies[15,16]. Hence, estimating GFR is very important in clinical nephrology and transplantation.

3.0 METHODS OF GFR ASSESSMENT:

3.1 DIRECT METHODS:

Direct methods include use of exogenous markers like Inulin, ^{131}I Iothalamate, ^{99m}Tc DTPA, ^{51}Cr EDTA, Iohexol and non-radiolabelled Iothalamate. Table 1 summarizes the characteristics of the direct markers of GFR.

Table 1 Characteristics of commonly used filtration markers:

Feature	Inulin	Iothalamate	DTPA	EDTA	Iohexol	Creatinine
MW (da)	5200	614	393	292	821	113
Elimination half-life (min)	70	120	110	120	90	200
Plasma protein binding (%)	0	< 5	5	0	< 2	0
Tubular secretion	-	+	-	-	-	++
Extra-renal elimination	-	-	+	+	-	+

3.1.1 INULIN / POLYFRUCTOSAN CLEARANCE:

Inulin Clearance is the gold standard method of evaluation of GFR. Inulin is a 5200MW polymer of fructose found in tubers like dahlia and chicory. It is an inert compound and is readily measured by colorimetric assays. Glucose is also detected in these assays and hence should be removed prior to the assay to prevent false positive results.

Homer Smith[17] originally developed the ***renal clearance*** method of measuring GFR. Patients are studied in the morning after an overnight fast. An oral water load of 10 to 15 ml /kg body weight is administered prior to infusion and additional water is administered throughout the test to ensure a constant urinary flow of 4 ml/min. Inulin is given as a ***constant infusion*** to achieve steady state concentration in plasma. Once steady state is achieved, several timed samples are taken. Ideally, the bladder needs catheterization. Serial plasma levels are also measured. The amount of inulin filtered at the glomerulus equals the GFR multiplied by the plasma inulin concentration (i.e., GFR × P_{in}). The amount of excreted inulin equals the urine inulin concentration (U_{in}) multiplied by the urine flow rate (V, volume excreted /unit time). Since

filtered inulin = excreted inulin

GFR X P_{in} = U_{in} X V

GFR = $(U_{in} \times V)/P_{in}$

The term $(U_{in} \times V)/P_{in}$ is defined as the clearance of inulin and is an accurate estimate of GFR. The inulin clearance, in ml/min, refers to the volume of plasma per unit time that is cleared of inulin by renal excretion. Since inulin is only filtered and neither secreted nor reabsorbed, all the inulin excreted is all the inulin that is filtered. This means the rate of inulin excretion is equal to the rate of inulin filtration. Therefore, the volume of plasma

cleared off inulin per unit time therefore indicates the volume of plasma that is filtered per unit time, which by definition is the GFR. Usually an average of three to five separate determinations has to be made. However, each of these measurements is subject to inaccuracies. The coefficient of variation between clearance periods is 5-10% and the coefficient of variation of inulin clearance measured on different days on the same individual is approximately 7.5%[18].

To avoid problems related to urine collection, many investigators have tested ***plasma clearance*** techniques. Plasma clearance of inulin can be measured with the use of either a constant infusion or a bolus injection[19]. If during a constant infusion both the distribution space and the plasma level of inulin are constant, the rate of infusion is equal to the rate of elimination. The inulin clearance therefore will be equal to the rate of infusion divided by the plasma concentration. There is a high degree of correlation between results from this technique and those from the renal clearance method[19]. However, maintaining constant plasma concentrations is subject to inaccuracies[20] and also very difficult[21]**.** Hence, the constant infusion technique is rarely used.

The measurement of GFR using a ***single injection technique*** is based on assumptions of critical importance. At any time, the quantity of indicator excreted by the kidney is d Q = GFR $\cdot$ C (t) d t where C (t) is the plasma concentration of the indicator at time t . When the all the marker injected (Q_0) has been excreted by the kidney, this equation gives:

$$\mathrm{GFR} = \frac{Q_0}{\int_0^{\infty} C(t)\,dt}$$

where the denominator represents the area under the plasma concentration–time curve. An accurate measurement of this parameter requires a large number of blood samples. Mathematical models have been developed to describe the decline of the marker plasma levels over time using a limited number of samples. All the models used to estimate plasma clearance assume that the volume distribution of the marker and its renal excretion are constant over time. One- and two-compartment models have been used to calculate GFR.

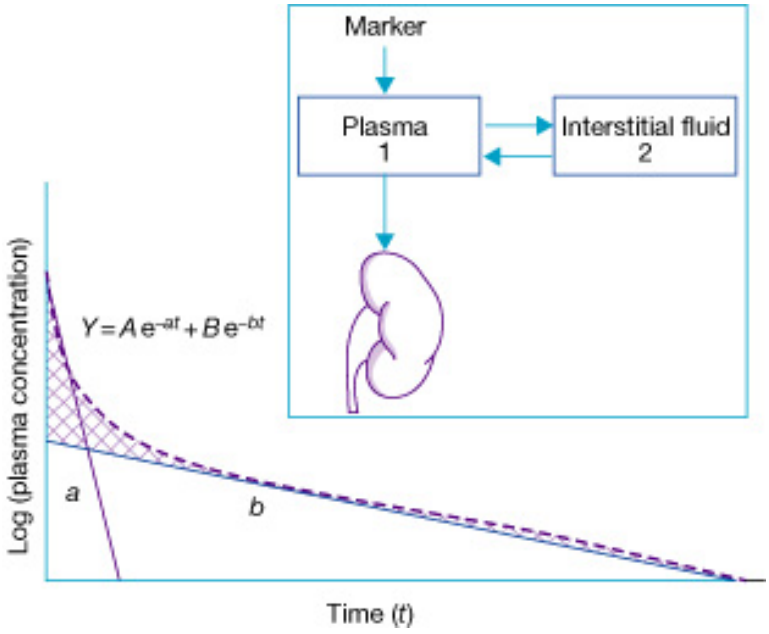


Figure1. The top panel represents a two-compartment model. The bottom panel shows a typical plasma disappearance curve after single intravenous injection of a marker of GFR. This curve can be expressed as a double exponential function. The first exponential corresponds to the marker equilibration between compartments 1 and 2, and the second function corresponds to the renal elimination phase. The straight lines represent the least-square best fit with slopes a and b, respectively. In a one-compartment model, the hatched area is not considered for calculating the GFR.

The plasma disappearance curve of the indicator can be fitted by the sum of two exponential functions of the form $C = A\,e^{-at} + B\,e^{-bt}$

This means that when plasma concentration is plotted on a logarithmic scale against time this curve appears as the sum of two straight lines with different slopes. These values can be substituted in the above equation to obtain the GFR:

$GFR = Q_0 / [(A / a) + (B / b)]$.

In the ***two-compartment model***, both slopes are used to calculate the area under the curve and the GFR. The first compartment can be thought of as the plasma pool and the second one as the extracellular fluid. Although the two-compartment model is oversimplified, since it assumes that the indicator distributes between only two pools, it still requires frequent plasma sampling. In this model, the first exponential corresponds to the equilibrium of the indicator between the two compartments and the second one represents its renal excretion.

In the ***one-compartment model***, only the data obtained during the elimination phase are used to calculate the second exponential and the GFR. This phase generally begins 120 min after injection. Since, in the one-pool system, the area under the curve is approximate, a correction is applied to obtain the GFR value. To ensure accurate measurements, ***multiple samplings*** are required to get the regression line during the elimination phase. Ideally, the higher the sample number the most accurate the value of the regression line and the calculation of the GFR. Alternatively, methods based on a ***single blood sample*** obtained between 60 and 240 min after injection can also be used. These techniques can give a reasonable estimate of the GFR. Nevertheless, multiple sampling yields a more accurate determination of GFR. Four samples drawn every hour

from 2 to 5 h after the indicator injection are enough in patients with normal or slightly decreased renal function (usually serum creatinine < 130 μM). When the decline of the GFR is more pronounced, the sampling time must be adapted. In these cases, samples obtained as late as 24 h after injection are suitable.

Another limitation to the use of plasma clearance technique is the presence of a ***third compartment***. For example, in patients with ascites the assumptions made to calculate the GFR from a one- or a two-compartment model are not met, so other mathematical models should be used

Thus, a number of problems limit the usefulness of inulin clearance as a marker of GFR. Although most data indicate that inulin is freely filtered and is not handled by the renal tubules, this indication may not be true for all clinical situations. For example, impaired filtration, back-diffusion of inulin, or both can limit the usefulness of this marker in kidney transplant recipients[22] . These logistic reasons along with the high cost and lack of availability have made inulin clearance at best a research tool rather than a clinical test.

The other substance that comes close to Inulin is polyfructosan. Nowadays the ***polyfructosans*** (Inutest® or Sinistrin®) are preferred to inulin since they are easily soluble in water at room temperature. These substances are not endogenously present in humans and must be intravenously infused throughout the GFR measurement. In practice, after a bolus injection, the dose of which is calculated according to the patient's body weight, the marker is infused at a constant rate to obtain a stable plasma concentration. This is achieved within 1h. To ensure a urine flow rate above 2 ml/min throughout the investigation, the patients are hydrated with an oral water load, and additional water is given periodically. Once the steady state for plasma marker concentration has been

achieved, the patients are asked to empty their bladder completely and several urine samples are collected at regular intervals (generally 30 or 60 min). The indicator plasma concentration is serially measured either at the middle or at the beginning and at the end of each period of urine collection. To minimize error, an average of three to five determinations are made. Polyfructosan and inulin determinations in the plasma are based upon hydrolysis of the polymer and measurement of the resultant free fructose with colorimetric assay. High-performance liquid chromatography (HPLC) techniques can also be used. Glucose may interfere with the colorimetric dosages, so plasma samples should be treated with glucose oxidase whenever plasma glucose concentrations are above 10 mM. The coefficient of variation of Polyfructosan or inulin clearance between consecutive periods or between measurements on different days is about 7%[18]. This means that for an initial GFR value of 40 ml/min, a variation of 6 ml/min in a subsequent investigation predicts a real modification of GFR at an alpha error less than 5%.

3.1.2 IOTHALAMATE CLEARANCE :

^{125} I-iothalamate is a high osmolar ionic radiocontrast agent, in which ^{127} I has been replaced by its isotope ^{125} I. Its MW is 614 Da. The relatively long half-life of ^{125} I (60 days) makes it easy to use. The main advantages of the radiolabelled indicators are the ease and the accuracy of measurements. Their drawback is the delivery of radioactivity. In adults, the total dose delivered is less than the amount received during standard X-ray procedures. However, these radioisotopes are excreted and concentrated in urine, exposing the bladder and the gonads to higher levels of radiation. Furthermore, in many patients, the measurement of GFR is controlled repeatedly throughout their lifetime

increasing the cumulative dose irradiation. The use of these radioactive markers is also problematic in women before menopause, since in most cases the physician cannot be sure that they are not pregnant. Similarly, the use of radioisotope in children should be avoided. In Europe and the United States, the regulatory agencies maintain a strict control over the storage, the dispensation, and the disposal of radioactive products and the use of isotopes is limited to specific departments.

To avoid using radiolabelled compounds, several techniques have been developed especially to measure non-radioactive radio-contrast agent concentration in plasma. Two molecules have been used: Iothalamate sodium, and more recently, Iohexol (omnipaque), which has become the most popular non-radioactive marker used to calculate GFR with the plasma clearance technique. Iothalamate estimation by HPLC is an alternative to radionuclide isotope method.

Renal clearance studies with Iothalamate has been shown to correlate well with GFR in most studies [2, 23 -26] ,,,. Standard renal clearance of Iothalamate using constant infusion to achieve steady state in blood can be considered as a relative gold standard technique of GFR estimation in the absence of Inulin. However, with a non renal correction factor of nearly 10ml/min/1.73sq.m, estimates of GFR by renal clearance with steady state infusion method and bolus injection method are comparable and the latter method is both precise and unbiased. Plasma clearance of Iothalamate is less accurate and overestimates GFR by about 3-10 ml/min/1.73sq.m[27].. Subcutaneous infusion methods have also been standardized in children for GFR estimation[28] .

3.1.3 DTPA CLEARANCE:

DTPA is an 110MW substance which can be tagged with Technetium radioisotope (^{99m}Tc) for use in nuclear medicine. GFR can be estimated either by a standard renal clearance or plasma clearance method or using the Renal Scintigraphy. The plasma clearance method is performed from whole plasma. However, in view of variable protein binding in hypoalbuminemia, which is frequently observed in the malnourished renal patient, it can also be performed from the protein-free ultra filtered plasma (PFP). Several methods of plasma clearance have been described. A comparative analysis[29] revealed that the two-sample plasma method of Russell and the urinary method of Jackson were the most accurate methods overall. The one-sample plasma method of Russell, the volume of distribution method of Fawdry, and a terminal slope method were less reliable, especially at low (0-60 ml/min) GFR. The Russell two point GFR and Jackson urinary GFR can be used as complementary techniques and are recommended as primary methods of DTPA GFR determination. Unlike the renal or plasma clearance techniques, in ^{99m}Tc-DTPA renography, the GFR is calculated without blood or urine sampling[30] . Several techniques have been applied in clinical practice, because of technical simplicity and requirement for less time for the patients. The method introduced by Gates[31] has been most common in the routine setting. When compared to the plasma clearance methods, the renal uptake method of Gates correlated poorly to the standards at all GFR levels even when corrected for body surface area or blood volume. In addition, Gates' method severely overestimated GFR in children[32] . Several modifications such as depth correction, use of dual gamma camera for scintigraphy and calculating geometric mean instead of arithmetic mean, have been reported [33] to improve the accuracy of the

gamma camera method for estimation of GFR. Although the diagnostic accuracy of the gamma camera methods is debated [34-37] ", the program is provided as a software package by manufacturers in commercially available computer systems dedicated for nuclear medicine. In addition, this method does not require sampling blood or urine for assessment of GFR. In spite of the shortcomings, the gamma camera method is still used widely in the clinical situation to assess GFR in many centers.

3.1.4 EDTA CLEARANCE:

Ethylenediaminetetraacetic acid (EDTA), a 292 MW substance with minimal protein binding capacity, when tagged with radioactive Chromium ^{51}Cr can be used for estimating GFR. It can be used with continuous infusion or bolus injection with single or multiple sampling methods for renal or plasma clearance methods for GFR measurement. ^{51}Cr EDTA is regarded as the standard radiopharmaceutical for routine GFR measurement in Europe. ^{51}Cr EDTA clearance from a single injection emerged as an adequate simpler technique in the early '70s[38] . GFR was calculated from the area under the plasma clearance curve, which required multiple blood samples to be taken over a period of several hours (Radio decay method). Although simpler than inulin infusion, it was still labor intensive and the technique was further simplified by restricting the blood sampling to the second of the two exponential components of clearance[39] (Slope-Intercept method). This simplification introduced systematic errors in the values of GFR obtained and various methods of correction have been derived[40] . The methodology underwent even further simplification by reducing the number of samples to one[41] . Empirical relationships between the apparent volume of distribution and GFR were

derived which allow GFR to be estimated from a single sample concentration with reasonable precision[42] . There are several systematic errors between the techniques. The DTPA clearance is systematically higher than that of EDTA[43] . EDTA plasma clearance is lower than inulin clearance. Urine clearance of EDTA is systematically less than that of plasma clearance[44] . The precision of EDTA clearance is more in range of normal GFR and hence it is useful for detection of hyperfiltration.

3.1.5 IOHEXOL CLEARANCE:

Iohexol (Omnipaque) is a 821 MW non ionic x-ray contrast medium of low osmolality that is extensively used in clinical radiology. It does not bind to serum proteins and is 100% filtered through glomerulus, with no indications of tubular secretion or reabsorption[45] . A good agreement was reported between the urinary clearance of inulin and the total body clearance of Iohexol over a wide range of GFR values[46] (6–160 ml/min/1.73sq.m.). Iohexol is measured by several methods such as HPLC (UV)[47] , capillary electrophoresis[48] , atomic spectroscopy[49] , chemical measurement (iodine), X-ray absorption and radionuclide scintigraphy. HPLC is commonly used for estimating the levels in plasma. It is extensively used in Sweden (>8000 GFR estimations)[50] . There is a strong concordance with inulin clearance. The Multiple sample method has fewer errors than single sample method.

3.1.6 CREATININE CLEARANCE:

Unlike the substances described until now Creatinine, a 113 MW substance is an endogenous marker of GFR. Creatinine is derived from the metabolism of creatine in

skeletal muscle[51] and from dietary meat intake[52] . Its production is reduced in liver disease[53] . It is released into the circulation at a relatively constant rate and has a stable plasma concentration. Creatinine is freely filtered across the glomerulus and is neither reabsorbed nor metabolized by the kidney. Hence, it is used as an *endogenous* marker. However, approximately 15 % of urinary creatinine is derived from tubular secretion through the organic-cation secretory-pathways in the proximal tubule[54] and this value may increase in settings of renal failure. The effect of secretion is usually ignored for day-to-day use. Then, all of the filtered creatinine (product of the GFR and the plasma creatinine concentration [P_{Cr}]) will be excreted (product of the urine creatinine concentration [U_{Cr}] and the urine flow rate [V]). Thus:

$$GFR \times P_{Cr} = U_{Cr} \times V$$

$$GFR = [U_{Cr} \times V]/P_{Cr}$$

This formula is more aptly called the ***creatinine clearance*** and tends to exceed the true GFR by the 10 to 15 % (due to the excess 10-15% of urinary creatinine that is derived from tubular secretion)[55] . Fortunately, this error is balanced by an error of almost equal magnitude in the measurement of the P_{Cr} in the laboratory. The creatinine clearance is usually determined from a 24-hour urine collection. Shorter collections tend to give less accurate results. The normal value for the creatinine clearance is 95 ± 20 ml/min in women and 120 ± 25 ml/min in men. However, creatinine clearance exceeds Inulin clearance at low GFR in view of tubular secretion. There is also a mild increase in extra – renal clearance of creatinine [51]. The ratio of urinary creatinine clearance to urinary inulin clearance may vary from 1.14 to 2.27 in different subjects.

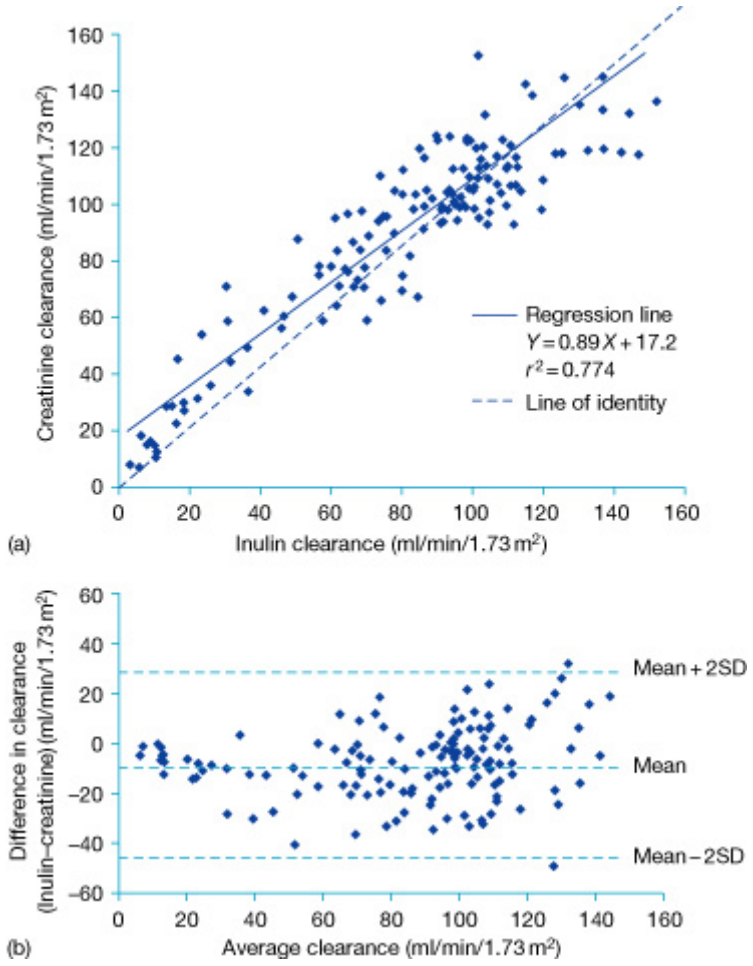


FIGURE 2. Comparison of the clearance of creatinine and inulin measured in 144 patients with normal or impaired glomerular filtration rate (Prié and Friedlander, unpublished data). Panel (a) shows the correlation of the two methods of measurement. Panel (b) shows the agreement of the two methods of measurements

The ***major errors*** limiting the accuracy of the creatinine clearance are:

- An incomplete urine collection
- Increasing creatinine secretion

An incomplete urine collection: The completeness of the collection can be estimated from knowledge of the normal rate of creatinine excretion (which is equal to creatinine production in the steady state). In adults under the age of 50, daily creatinine excretion

should be 20 to 25 mg/kg (177 - 221 µmol/kg) of lean body weight in men and 15 to 20 mg/kg (133 - 177 µmol/kg) of lean body weight in women. From the ages of 50 to 90, there is a progressive decline in creatinine excretion to 50 % (to about 10 mg/kg in men), primarily due to a fall in muscle mass.

Increasing creatinine secretion: The accuracy of the creatinine clearance is also limited by the fact that enhanced creatinine secretion limits the rise in P_{Cr} when the GFR falls [56]. This secretory capacity is also not uniform. For example, when the GFR falls to a range of 40 to 80 ml/min (as measured by alternative modalities), the absolute amount of creatinine secreted can rise by more than 50 %, accounting for as much as 35 % of urinary creatinine [24]. Thus, creatinine excretion is much greater than the filtered load, resulting in a potentially large overestimation of the GFR. The net effect is that the creatinine clearance may be normal (>90 ml/min) in about one-half of patients with a true GFR of 61 to 70 ml/min and one-quarter of those with a true GFR of 51 to 60 ml/min[57]. Some patients with advanced disease have a creatinine clearance that exceeds the GFR by more than twofold. Thus, creatinine clearance represents an upper limit of what the true GFR may be.

Drugs like trimethoprim and cimetidine compete and block the pathways of creatinine excretion in the renal tubules. Oral administration of Cimetidine at a dose of 400mg BD to a total cumulative dose of 1200mg, results in complete blockade of the tubular secretion of creatinine in most patients. Thus the creatinine that is excreted is derived exclusively from the glomerular filtration[58, 59]. These methods improve the accuracy

(bias and precision) of creatinine clearances[60, 61] , . However, some subjects required relatively large doses for complete inhibition of renal tubular secretion of creatinine.

In early renal disease when the GFR is still near normal, an initial decline in GFR may lead to only a slight increase in the serum creatinine (S.Cr) (0.1 to 0.2 mg/dl [9 to 18μmol/L]). This is because of an increase in proximal tubular creatinine secretion. The net effect is that patients with a true GFR as low as 60 to 80 ml/min (as measured by the clearance of a true filtration marker such as inulin, Iothalamate or DTPA) may still have a S.Cr that is ≦1.0 mg/dl (88 μmol/L)[62] . Thus, a relatively stable S.Cr in the normal or near-normal range does not necessarily imply that the disease is stable. However, once the S.Cr exceeds 1.5 to 2 mg/dl (132 to 176 μmol/L), the secretion is effectively saturated. Hence, a stable value above that usually does represent a stable GFR.

Limited data also suggests that tubular secretion is significant in patients with the nephrotic syndrome. In one study based upon a determination of GFR by inulin clearance, decreased serum albumin levels were associated with a marked increase in tubular creatinine secretion (36 ml/min /1.73 Sq.m for nephrotic patients with serum albumin levels less than 2.6 g/dl versus 11 ml/min /1.73 Sq.m for normal controls)[63] .

The degree of creatinine secretion may vary with time, affecting the S.Cr independent of the GFR. In effectively treated lupus nephritis, for example, a rise in the GFR may not be accompanied by the expected reduction in the S.Cr due to a fall (via an uncertain mechanism) in creatinine secretion[64] . In this setting, decreased activity of the urine sediment, diminished protein excretion, and lack of further elevation in the S.Cr all point toward possible improvement.

There are certain settings in which there may be an acute increase in creatinine production. One example is a recent meat meal. In addition, it has been suggested that the S.Cr rises more rapidly with rhabdomyolysis (up to 2.5 mg/dl or 220 µmol/L /day) than with other causes of acute renal failure[65] .

The presence of certain drugs may increase the plasma level of the serum creatinine by decreasing creatinine secretion. This includes trimethoprim (most often given in combination with sulfamethoxazole) and the histamine 2-blocker cimetidine, which result in a self-limited and reversible rise in the S.Cr of as much as 0.4 to 0.5 mg/dl (35 to 44 µmol/l). Certain substances may interfere with the plasma assay; thereby artifactually increasing the S.Cr Value.

Direct Clearance methods thus provide an acceptable measure of GFR, though there is a tendency to overestimate GFR by about 3.5-8ml/min/1.73sq.m. However, the procedure is cumbersome and costly for routine clinical use. However, it may be of value in certain clinical situations such as:

1. Extremes of body size
2. Extremes of age (low BMI for MDRD formula)
3. High or low dietary intake of creatinine (dietary supplements, vegans/vegetarians)
4. Patients with muscle diseases or atrophy (muscular dystrophy, amputation, paralysis, malnutrition)
5. Particular ethnic groups (Aborigines, Pacific Islanders, Indo-Asians)

3.2 INDIRECT METHODS:

For many years, the physicians have been seeking for an endogenous marker, the plasma concentration of which would be related to the renal filtration levels and would be readily measured. For this purpose, three molecules have been used: urea, creatinine, and CysC.

3.2.1 Urea:

Urea was the first described marker of renal function. Even today renal failure is still referred to as uremia. Urea is a small molecule (60MW) derivative of nitrogen metabolism in the liver. Daily urea production varies not only with protein intake but also dependent on intestinal protein breakdown. Conditions where intestinal protein catabolism is high, such as gastrointestinal bleeding, Plasma Urea levels are elevated even with normal renal function[66] . Urea is freely filtered by the glomeruli. However, it is easily reabsorbed by the tubules, mostly in PCT, in parallel with Na and water reabsorption. Hence, several factors (apart from decreased GFR) modify plasma urea and urea renal excretion[67] . In conditions when water and sodium reabsorption is elevated, such as in hypovolemia or hypotension, urea reabsorption is augmented leading to high urea serum concentration (pre renal azotemia). Adversely, liver diseases reduce urea plasma levels. For these reasons, plasma urea cannot be used for the assessment of the GFR[68] .

3.2.2 Creatinine:

Serum creatinine concentration (S.Cr) is dependent on GFR and was first employed as a marker of GFR in clinical medicine[69] in early ‘20s. Creatinine excretion (GFR x S.Cr)

equals creatinine production in the steady state and that creatinine production is relatively constant. Thus GFR x S.Cr = constant. Thus, the plasma creatinine concentration varies inversely with the GFR (Figure 3.) If, for example, the GFR declines by about 50%, the creatinine excretion will initially be reduced, leading to creatinine retention and a rise in the S.Cr until the latter has doubled. At this point, the filtered load will again be equal to excretion:

GFR/2 x 2 S.Cr = GFR x S.Cr = Constant

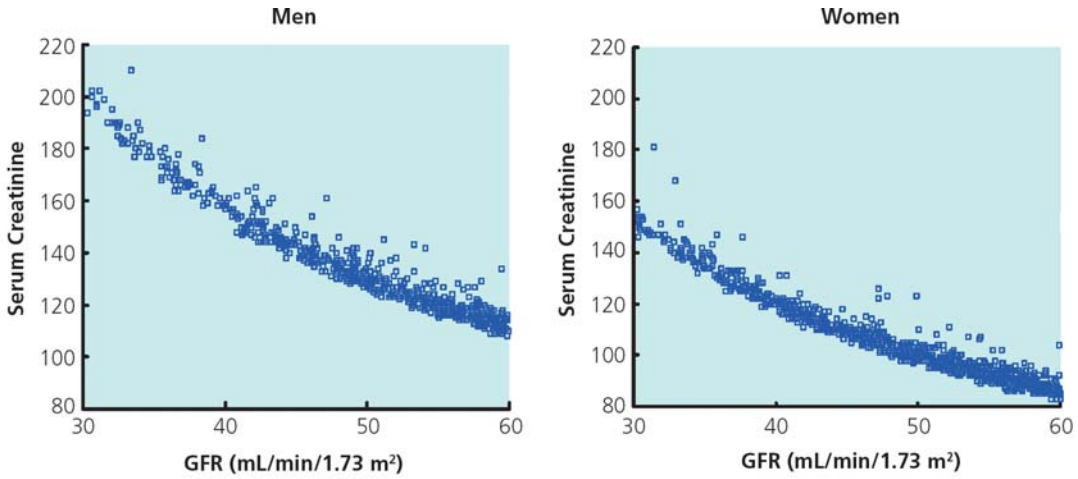


Figure 3. Relationship between plasma creatinine and GFR.

A rising S.Cr implies disease progression, a falling level indicates improvement, and a stable value usually reflects stable disease. Nevertheless, there are several factors that might influence plasma creatinine (see *Table 2*)

Based on the third *National Health and Nutrition Examination Survey*, among individuals without hypertension or diabetes in the United States, the mean S.Cr values for men and women[70] were 1.13 and 0.93 mg/dl (100 and 82 µmol/L), respectively. There are several **limitations with plasma creatinine values**.

Table 2. Factors affecting the Serum creatinine levels

Table 1: Factors affecting serum creatinine concentration		
	Effect on Serum Creatinine	**Mechanism/Comment**
Age	Decrease	Reduction in creatinine generation due to age-related decline in muscle mass
Female Sex	Decrease	Reduced creatinine generation due to reduced muscle mass
Race		
African American	Increase	Higher creatinine generation rate due to higher average muscle mass in African Americans; not known how muscle mass in other races compares to that of African American or Caucasians
Hispanic	Decreased	
Asian	Decreased	
Diet		
Vegetarian Diet	Decrease	Decrease in creatinine generation
Ingestion of Cooked Meats	Increase	Transient increase in creatinine generation; however, this may be blunted by transient increase in GFR
Body Habitus		
Muscular	Increase	Increased muscle generation due to increased muscle mass ± increased protein intake
Malnutrition/muscle wasting/amputation	Decrease	Reduced creatinine generation due to reduced muscle mass ± reduced protein intake
Obesity	No Change	Excess mass is fat, not muscle mass and does not contribute to increased creatinine generation
Medications		
Trimethoprim, cimetidine, fibric acid derivatives other then gemfibrizol	Increase	Reduced tubular secretion of creatinine
Keto acids, some cephalosporins	Increase	Interference with alkaline picrate assay for creatinine

The Jaffe's alkaline picrate method (colorimetric) of S.Cr assay recognizes non-creatinine chromogens (e.g. acetoacetate)[71] , thereby falsely elevating S.Cr values by ≥ 0.5 - 2 mg/dl. Modern auto analyzers use S.Cr assays with less interference by chromogens (e.g., kinetic alkaline picrate or enzymatic methods, such as the imidohydrolase method). Laboratories with calibration differences can provide varying S.Cr measurements that can lead to erroneous estimations of GFR in patients with relatively normal S.Cr[72] . Differences in method and equipment may also affect creatinine measurement.

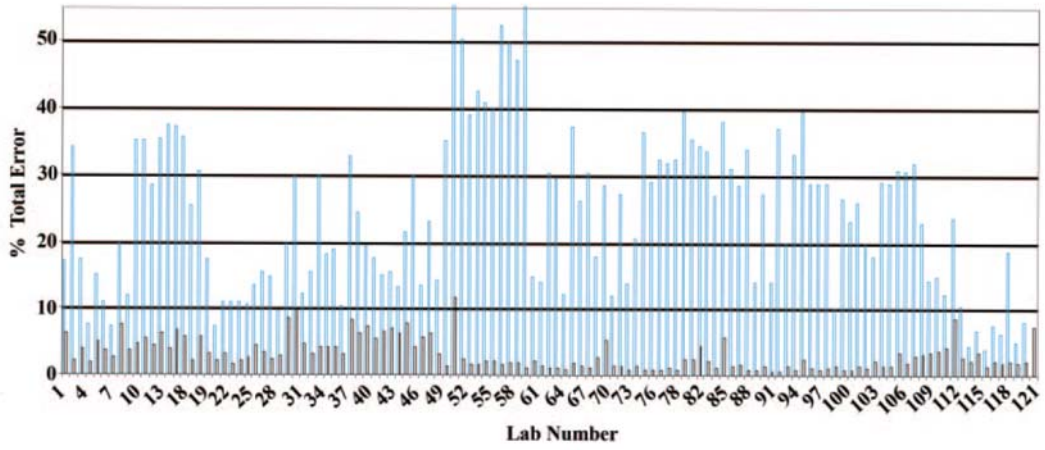


Figure 4. The Creatinine Standardization Program, Canada. 107 laboratories, which tested creatinine on 124 analyzers from six different manufacturers, were analyzed. The percentage total error for the measurement of creatinine (98.9 µmol/L) at baseline is depicted as blue bars (ranging from 4 to 54% with an overall average of 23.9%). The between-day precision (coefficient of variance) is also plotted (black bars).

When the S.Cr > 6 mg/dl (530 µmol/L), there is intestinal bacterial overgrowth and increased bacterial creatininase activity[73] , resulting in increased extra renal creatinine clearance, reduction in S.Cr and overestimation of GFR.

In summary, S.Cr is affected by the level of GFR and by factors independent of GFR, including age, gender, race, body size, and diet, certain drugs, and laboratory analytical methods. Therefore, S.Cr is not an accurate index of the level of kidney function, and the level of S.Cr alone should not be used to assess the stage of chronic kidney disease. However, since S.Cr can be easily measured routinely, many authors have tried to find mathematical formulae taking into account the various causes of variation of serum creatinine to deduce the GFR level.

The Creatinine Based Estimated GFR formulae:

Prediction equations for GFR calculation from S.Cr have been developed by several authors. There are nearly 45 prediction equations in literature.

Table 3. Prediction Equations for eGFR based on Serum Creatinine

Reference	Formulae
S_{cr}-based equations	
Cockcroft–Gault[a]	$\frac{(140-\text{Age})}{72\times S_{cr}}(\text{Wt}) \times (0.85 \text{ for female})$
Bjornsson[a]	For males: $\frac{[27-(0.173\times\text{Age})]\times\text{Wt}\times 0.7}{S_{cr}}$
	For females: $\frac{[25-(0.175\times\text{Age})]\times\text{Wt}\times 0.7}{S_{cr}}$
Davis[a,c]	$\frac{140-\text{Age}}{S_{cr}} \times (0.85 \text{ for female})$
Edwards[a,c]	For males: $\frac{94.3}{S_{cr}} - 1.8$
	For females: $-\frac{69.9}{S_{cr}} + 2.2$
Gates[a]	For males: $89.4 \times S_{cr}^{-1.2} + (55 - \text{Age}) \times 0.447 \times S_{cr}^{-1.1}$
	For females: $60 \times S_{cr}^{-1.1} + (56 - \text{Age}) \times 0.3 \times S_{cr}^{-1.1}$
Hull[a]	$\frac{(145-\text{Age}-3)}{S_{cr}} \times (0.85 \text{ for female})$
Jelliffe[a]	$\frac{(98-0.8(\text{Age}-20)}{S_{cr}} \times (0.90 \text{ for female})$
Jelliffe[a]	For males: $\frac{100}{S_{cr}} - 12$
	For females: $\frac{80}{S_{cr}} - 7$
Mawer[b]	For males: $\frac{\text{Wt}\times(29.3-0.203\times\text{Age})\times[1-(0.03\times S_{cr})]}{(1.44\times S_{cr})\times(70/\text{Wt})}$
	For females: $\frac{\text{Wt}\times(25.3-0.175\times\text{Age})\times[1-(0.03\times S_{cr})]}{(1.44\times S_{cr})\times(70/\text{Wt})}$
MDRD2[a]	$186 \times (\text{Scr})^{-1.145} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African} - \text{American})$
MDR 2[a,d] (IDMS)	$175 \times (\text{Scr})^{-1.145} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African} - \text{American})$
Nankivell[b]	$(6.7/\text{Scr}) + (\text{Wt}/4) - (\text{Urea}/2) - (100/\text{height}^2) + [35 \text{ for male or } 25 \text{ for female}]$
Salazar[a]	For males: $\frac{(137-\text{Age})\times[(0.285\times\text{Wt})]+(12.1\times\text{Height}^2)}{(51\times\text{Scr})}$
	For females: $\frac{(140-\text{Age})\times[(0.285\times\text{Wt})]+(12.1\times\text{Height}^2)}{(60\times\text{Scr})}$
Walser[b,e]	For male: $\frac{7.57}{S_{cr}} - 0.103 \times \text{Age} + 0.096 \times \text{Wt} - 6.66$

These equations include variables such as age, sex, race, and body size, in addition to serum creatinine, as surrogates for muscle mass, for calculation of GFR. They are all derived with the use of regression techniques to model the observed relation between the S.Cr and the measured GFR in a study population. However, most of the equations have been studied in restricted populations - usually patients with CKD and reduced GFR. Hence, evaluation of these equations in other populations is necessary to demonstrate the generalizability. A few of these equations are described in detail:

1) The Cockcroft-Gault GFR:

The Cockcroft-Gault formula was originally derived in 249 consecutive hospitalized patients (96% male, age range 18–92 years) at the Queen Mary Veterans' Hospital in Canada, based on the means of two 24-hour creatinine clearances[3]. S.Cr concentrations were determined by Jaffé reaction using an auto analyzer (N-11B, Technicon Instruments Corp, NY). The Cockcroft- Gault Formula:

CrCl = {[(140-age) X weight] / (72 X S.Cr)} X (0.85 if female)

The derived formula was then used to predict creatinine clearance in a validation cohort of 236 patients (206 males, mean creatinine clearance 72.7 ± 36.6 ml/min). Mean predicted creatinine clearance by the Cockcroft-Gault formula was 75.8 ml/min with an R^2 of 0.69. Accuracy at ≤35% and ≤20% was observed in 95% and 67% of patients respectively. The main limitations of the study were:

1. The questionable external validity, as the training and validation samples consisted predominantly of hospitalized, white men, many of whom did not have CKD. Nevertheless, the Cockcroft-Gault formula has been extensively validated and found to exhibit satisfactory accuracy in diverse populations including women, various ethnic groups, and across a broad range of GFRs. These studies are listed in Table 4.
2. The formula was originally validated against creatinine clearance, which is known to appreciably overestimate inulin clearance and vary from day to day by 10%–20%[74] . Subsequent validation studies have generally shown equivalent or superior performance against a variety of GFR measures (inulin, iothalamate, 51Cr-EDTA, DTPA, MAG-III and Iohexol clearances).
3. The calibration bias of creatinine assay used vs. 'true' creatinine was not reported.

4. The results of the Cockcroft-Gault formula were not corrected for body surface area.

Table 4. Studies evaluating the Cockcroft – Gault GFR formula

Author, Year Equation/Sample	No. of Subjects* (Measurements)	Applic-ability	GFR Range (mL/min/1.73 m²) 0 30 60 90 120	Accuracy** 30%	50%	Bias† (%)	Quality
Lewis,[162] 2001[a]	1,775	††		80	94	1	●
Levey,[17] 1999	1,070/558[c]	†††					●
BSA corrected CG[a]				65	83	23	
Bias corrected CG[a,b]				81	94	4[b]	
Rolin,[159] 1984	394 (500)	†††					●
Uncorrected CG				73[d]	91	10	
BSA corrected CG[a]				81[d]	96	3	
Toto,[135] 1997[a]	193	††		78	98	−14	●
Lemann,[160] 1990		††					●
Diabetics	136			77	92	1	
Healthy & stone formers	110			96	100	−8	○
Charleson,[161] 1980	100	†††		66	84	—	○
Waller,[163] 1991	171	††		81	94	0	○
DeSanto,[164] 1991	124	††		70	85	—	○
CKD	62			48	70	−5	
Healthy	62			92	100	0	
Gault,[120] 1992	100 (197)	††		75	92	5	○
Bedros,[165] 1998	321 (708)	††		68	84	13	○
Bias corrected CG[b]				76	93	0[e]	
Goerdt,[166] 1997	127 (142)	††		66	85	25[e]	○

The Cockcroft-Gault equation is: $C_{Cr}\,(\text{ml/min}) = \dfrac{(140 - Age) \times Weight}{72 \times S_{Cr}} \times (0.85 \text{ if female})$

Markers for measuring GFR include ^{125}I-iothalamate (Levey[17], Rolin[159], Toto[135], Lemann (diabetics)[160], Lewis[162]), Inulin (Lemann (healthy)[160], De Santo[164]), ^{99m}Tc-DTPA (Waller[163], Gault[120]), ^{51}Cr EDTA (Charleson[161]), Iohexol (Goerdt[166]).

* When multiple measurements were made for each subject, the total number of measurements appears in parentheses.
** Accuracy defined as the percent of GFR estimates within 30% or 50% of measured GFR.
† See Part 10, Appendix 3 for definition of bias.

[a] Cockcroft-Gault equation standardized for 1.73 m^2 body surface area
[b] Bias correction utilized a multiplier which corrected the overall bias of the development set to 0. The bias noted is calculated from the validation (test) set when available.
[c] 1,070 subjects in model development set; 558 subjects in validation set.
[d] Percent of estimates within 35% of the true value.
[e] Bias is rough estimation from the graph.

Abbreviations: CKD, chronic kidney disease; CG, Cockcroft-Gault Equation, BSA, body surface area

The bias in estimating GFR using the Cockcroft-Gault equation varied markedly across studies (from -14% to +25%). The accuracy measures indicated the majority (median of 75%) of estimated GFRs were within 30% of the measured GFR, an accuracy considered sufficient for good clinical decision-making. The Cockcroft-Gault equation does not include body size. Some studies have standardized the results for body surface area. Other studies have suggested using lean body mass[75] rather than total weight, especially for obese individuals.

2) Modification of Diet in Renal Disease (MDRD) GFR Formulae:

The MDRD equation was developed in 1628 CKD patients enrolled in the baseline period of the Modification of Diet in Renal Disease (MDRD) study, of whom 1070 were randomly selected as the derivation sample and the remaining 558 patients constituted the validation sample[4]. The exclusion criteria for the MDRD study were patients with body weight extremes (< 80% or > 160% of standard body weight), dubious compliance, insulin-dependent diabetes mellitus or heavy proteinuria (> 10 g/day). GFR was directly measured as iothalamate clearance and S.Cr was measured by means of a modified kinetic Jaffé reaction using a Beckman Astra CX3 autoanalyzer (Brea, CA). Using multiple regression analysis, a 6-variable equation (**equation 7 – MDRD 1**) was developed, which included the variables of S.Cr, age, gender, ethnicity (African-American or other), serum urea and serum albumin. The MDRD- 1 equation is:

$$\textbf{MDRD-1 GFR} = 170 \times (\text{S.Cr})^{-0.999} \times \text{age}^{-0.176} \times (\text{Ur} \times 2.78)^{-0.17} \times \text{alb}^{0.318} \times (0.762 \text{ if female}) \times (1.18 \text{ if African-American})$$

The equation was validated against GFR corrected for body surface area (BSA) and so, unlike the Cockcroft-Gault formula, the predicted GFR is expressed as ml/min/1.73sq.m, and does not require subsequent BSA normalization. Compared with the BSA-corrected Cockcroft-Gault formula, the MDRD-1 equation was more precise (r^2 0.90 vs. 0.84), less biased (3% vs. 23%) and exhibited greater accuracy within 30% (91% vs. 65%) and within 50% (98% vs. 83%). The accuracy of GFR values was worst for Cockcroft-Gault GFR (non- BSA-corrected), intermediate for BSA-corrected GFR, and best for MDRD GFR[76] . The precision, accuracy and bias of the MDRD GFR has been validated in 5069 subjects over 16 studies and generally found to be superior to those of the Cockcroft-Gault formula.

An abbreviated, 4-variable MDRD equation (age, gender, African-American ethnicity and S.Cr) has subsequently been developed (**MDRD-2 or Abbreviated MDRD Formula**). The Abbreviated MDRD Formula for creatinine evaluated by Jaffe method is

$$\textbf{MDRD2 GFR} = 186 \times (\text{S.Cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})$$

However, with recent efforts at standardizing the serum creatinine measurement to avoid analytical errors, some laboratories measure S.Cr values standardized to the IDMS (isotope dilution mass spectrometry) reference method. A new MDRD 2 formula has been developed for use with IDMS traceable S.Cr measurement:

$$\textbf{Std. MDRD2 GFR} = 175 \times (\text{S.Cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})$$

The MDRD2 GFR has been validated in 8654 subjects over 13 studies.

Table 5. Studies evaluating the MDRD GFR formulae

Table 47. Estimating GFR in Adults Using the MDRD Study Equation: Accuracy and Bias

Author, Year Equation	No. of Subjects* (Measurements)	Applicability	GFR Range (mL/min/1.73 m²)	Accuracy** 30%	Accuracy** 50%	Bias† (%)	Quality
Levey,[17] 1999 Levey,[18] 2000	1,070/558[e]	†††	0 30 60 90 120				●
Equation 1[a]				91	98	3	
Equation 2[b]				92	98	3	
Equation 3[c]				91	98	3	
Equation 4[d]				91	98	3	
Lewis,[182] 2001	1,775	††					○
Equation 1[a]				88	97	1	
Equation 2[b]				88	98	−1	
Equation 3[c]				88	98	−1	
Equation 4[d]				88	98	−3	
Bedros,[185] 1998 Equation 4[d]	321 (708)	††		84	97	−1	○

* When multiple measurements were made for each subject, the total number of measurements appears in parentheses.
** Accuracy defined as the percent of GFR estimates within 30% or 50% of measured GFR.
† See Part 10, Appendix 3 for definition of bias.

[a] Equation 1: $GFR = 170 \times (S_{Cr})^{-0.999} \times (Age)^{-0.176} \times (BUN)^{-0.170} \times (Alb)^{+0.318} \times (0.762 \text{ if female}) \times (1.180 \text{ if African-American})$ ("Equation 7" in Levey,[17] 1999)

[b] Equation 2: $GFR = 198 \times (S_{Cr})^{-0.858} \times (Age)^{-0.167} \times (BUN)^{-0.293} \times (UUN)^{+0.249} \times (0.822 \text{ if female}) \times (1.178 \text{ if African-American})$ ("Equation 6" in Levey,[17] 1999)

[c] Equation 3: $GFR = 270 \times (S_{Cr})^{-1.007} \times (Age)^{-0.180} \times (BUN)^{-0.169} \times (0.755 \text{ if female}) \times (1.178 \text{ if African-American})$ ("five-variable" equation in Levey,[18] 2000)

[d] Equation 4: $GFR = 186 \times (S_{Cr})^{-1.154} \times (Age)^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African-American})$ ("four-variable" (abbreviated) equation in Levey,[18] 2000)

[e] 1,070 subjects in model development set; 558 subjects in validation set.

Abbreviations and units: GFR, glomerular filtration rate in mg/mL/1.73 m²; S_{Cr}, serum creatinine in mg/dL; age, in years; weight in kg; BUN, blood urea nitrogen in mg/dL; Alb, serum albumin in g/dL; UUN, urine urea nitrogen

It was generally found to be comparable to those of MDRD1 GFR and superior to those of Cockcroft-Gault GFR. Several studies have confirmed that the MDRD2 equation shows generally greater precision and accuracy than the Cockcroft-Gault formula in patients with CKD (GFR < 60 ml/min/1.73 Sq.m)[77-79] , but tends to be more biased with significant underestimation of measured GFR in patients with normal or near-normal renal function[80-83] . In view of wide range of applicability, better precision and accuracy in lower GFR ranges, The National Kidney Foundation[1] and the European Renal Association[84] have endorsed this formula for diagnosis, classification and management of Chronic Kidney Disease.

3) Nankivell GFR Formula:

Nankivell et al (1995) developed a new prediction equation for estimating GFR in renal transplant recipients[85] based on a total of 256 randomly selected DTPA GFR measurements. Three new formulae were derived and then tested on an independent group of randomly selected GFR measurements (n = 255), comparing with the Cockcroft-Gault, Mawer[86] , Hull[88] , Jelliffe-1[88] , and Jelliffe-2[89] methods. The Formula B was found to provide better precision and less scatter and was proposed to replace other published formulae for calculating GFR in renal transplant recipients.

Formula B Nankivell GFR = [6700 / (S.Cr X 88.4)] + (weight/4) - (urea/12)

– [100/(height)^2] + 35 – [10 (if female)]

The major weakness of this study was that the prediction equations were not tested in a large independent cohort, making the external validity of this study questionable. The proportion of predicted GFR differing from inulin clearance by ±10 ml/min/1.73 Sq.m, was highest for Nankivell formula compared to MDRD and Cockcroft Gault GFR formulae[90] . Overall, the current evidence suggests that the MDRD formula is the best available equation for estimating GFR in transplant recipients.

4) Mayo Clinic Quadratic GFR Formula:

Rule et.al. from Mayo Clinic, analyzed a series of 580 potential kidney donors referred to the Mayo Clinic and found that the MDRD-2 equation significantly underestimated GFR in healthy persons. This led the authors to develop new equations that performed better than the abbreviated MDRD formula in healthy persons.

Table 6. The Mayo Clinic Quadratic Equation for GFR

Equation 1: Original MDRD equation (1628 patients with chronic kidney disease) (4)

$$GFR = 186 \times SCr^{-1.154} \times Age^{-0.203} \times 0.742 \text{ (if female)} \times 1.21 \text{ (if black)}$$

Equation 2: Refit MDRD equation (320 predominantly white patients with chronic kidney disease)

$$GFR = 297 \times SCr^{-1.290} \times Age^{-0.290} \times 0.767 \text{ (if female)}$$

Equation 3: Refit MDRD equation (580 predominantly white healthy persons)

$$GFR = 216 \times SCr^{-0.490} \times Age^{-0.192} \times 0.923 \text{ (if female)}$$

Equation 4: Refit MDRD equation with healthy indicator variable (320 patients with chronic kidney disease and 580 healthy persons)

$$GFR = 224 \times SCr^{-1.190} \times Age^{-0.236} \times 0.796 \text{ (if female)} \times 1.26 \text{ (if healthy)}$$

Equation 5: Quadratic GFR equation (320 patients with chronic kidney disease and 580 healthy persons)

$$GFR = \exp\left(1.911 + \frac{5.249}{SCr} - \frac{2.114}{SCr^2} - 0.00686 \times Age - 0.205 \text{ (if female)}\right)$$

If $SCr < 0.8$ mg/dL, use 0.8 for SCr

* GFR = glomerular filtration rate (mL/min per 1.73 m^2); MDRD = Modification of Diet in Renal Disease; SCr = serum creatinine (mg/dL). Age is in years. Numbers in parentheses are the population used to derive the equation.

In a subsequent study among elderly adults from the community[91] , it was found that using the MDRD2 equation increased the prevalence of CKD and weakened the epidemiologic association between GFR and mortality. However, the Mayo Quadratic equation (derived by using healthy persons and patients with chronic kidney disease) decreased the prevalence of a CKD and strengthened association with mortality in the same population. Hence, the authors concluded that in order to better understand the epidemiology of early CKD, Mayo Quadratic GFR may be better than MDRD2. These equations however have not significantly outperformed MDRD-2 equation in other studies[92] and tend to classify more people as suffering from CKD.

3.2.3 Cystatin C:

Cystatin C (CysC) is a 122-amino acid, 13-kDa protein member of cysteine proteinase inhibitors. CysC has been suggested to be closer to the "ideal" endogenous marker[93] owing to the several desirable properties:

1. A constant rate of production by a 'housekeeping' gene in all nucleated cells[94] ,
2. Lack of effect of age, gender or muscle mass on CysC production[95-97]
3. Free filtration at the glomerulus because of its small size and basic pI (~9.0)[98] ,
4. Complete reabsorption and catabolism by the proximal tubule cells[99] , (hence, cannot be used for urine clearance techniques)
5. Lack of renal tubular secretion[98],
6. Lack of reabsorption back into the bloodstream[98], and
7. Minimal or no analytical interference[100] .

The diagnostic value of serum CysC in clinical nephrology was not extensively investigated until 1994 because of general difficulties in standardizing immunometric methods. More recently, automated homogeneous immunoassays using latex or polystyrene particles coated with CysC-specific antibodies have been developed based on turbidimetry (particle-enhanced turbidimetric immunoassay, PETIA) [100] or nephelometry (particle-enhanced nephelometric immunoassay, PENIA)[101] . The PETIA method generally produces reference values that are 20%–30% higher than those from the PENIA method. A meta-analysis demonstrated that the correlation between GFR and the reciprocal of CysC was significantly stronger when CysC was measured by the PENIA method (14 studies including 1698 subjects) rather than when it was measured by other methods (21 studies involving 1953 subjects)[102] . In this meta-analysis (of 54 studies,

incorporating 4492 subject samples of CysC as a GFR index), it was observed GFR correlated significantly better with the reciprocal of CysC (r = 0.816, 95% CI 0.804–0.826) than with the reciprocal of S.Cr (r = 0.742, 95% CI 0.726–0.758, p < 0.001). Thus, CysC was clearly superior to S.Cr as a marker of GFR.

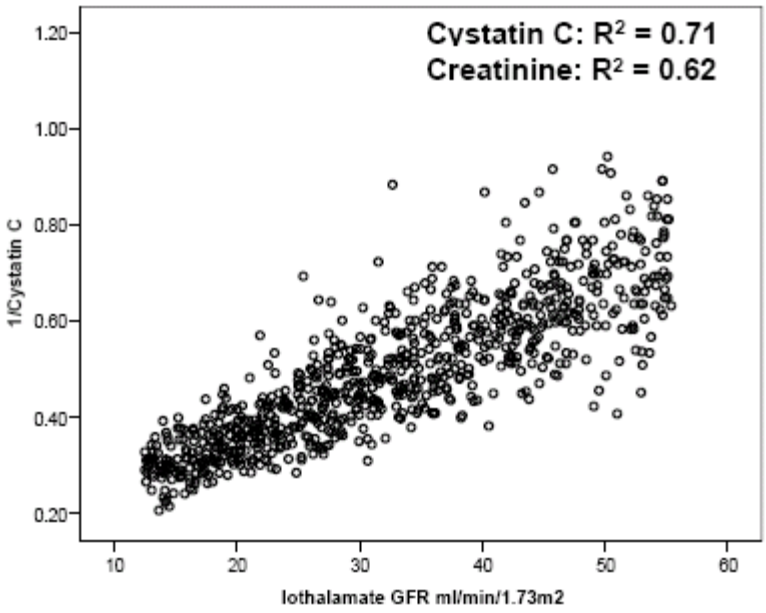


Figure 5. Cystatin C values correlate well with GFR

However, this finding must be balanced against the potential limitations of the studies of CysC as a GFR marker to date, which include

a. Lack of standardization (differences in antibodies, calibrators & technologies)[103]

b. Biological variability of CysC (PETIA[104] vs. EIA[105]) – [Variability %: analytical - 8.9% vs. 8.8%, within-subject - 13.3% vs.15% and between-subjects -8.1% vs. 5.7%.]

c. Factors influencing CysC levels independent of creatinine clearance such as: age, weight[106], gender, CRP level, smoking[107], cyclosporine A[108], corticosteroid treatment[109], bronchial asthma[110], thyroid dysfunction[111, 112], physical activity[113], certain malignancies[114] and pregnancy[115].

d. Lack of standardization of statistical analyses,

e. Absence of wide applicability like MDRD-2 GFR

The data form the biological variability suggested that CysC may be better at detecting the onset of an abnormal GFR than S.Cr, and equally (if not more) sensitive as S.Cr for detecting changes in the same individual. The sensitivity of CysC is higher at higher GFR ranges compared to S.Cr. A recent study[116] has confirmed that CysC is better predictor of longitudinal changes in GFR than S.Cr. (MDRD).

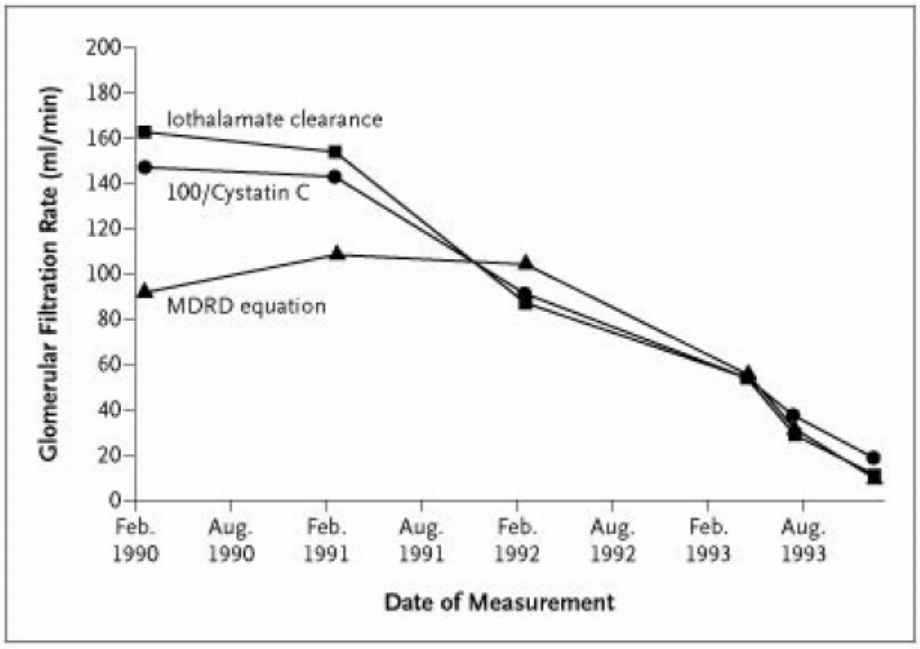


Figure 6. CysC is better predicts longitudinal changes in GFR

Some studies have shown that CysC may be falsely elevated in renal transplant patients leading to a significant underestimation of GFR by 14%–25%[117], possibly because of interference by immunosuppressive agents: corticosteroids can increase CysC levels whereas cyclosporine decreases CysC concentrations. In several studies, CysC levels correlated well with mortality[118] and cardiovascular risk[119].

The Cystatin C Based Estimated GFR formulae:

Several prediction equations for estimating GFR from CysC values have been devised. We will discuss a few of them in detail here:

1) Le Bricon GFR [120]:

The formula was derived from a population of 25 renal allograft recipients, who were followed up for at least 3 months duration. CysC was measured by the Dade Behring PENIA method. Reference GFR was measured by GFR by the reference ^{51}Cr EDTA clearance. GFR could be estimated from CysC (in mg/L) according to the following formula:

Le Bricon GFR (ml/min/1.73sq.m) = 78 X (1/CysC) + 4

Overall, CysC underestimated GFR by 14% with no false negatives.

2) Larsson GFR[121]:

This formula was developed separately for the CysC measured by Dade Behring PENIA and Dako Cytomation PETIA methods, from 100 patients (adults and children) referred to a Swedish center for Iohexol clearance (Reference GFR). The formulae were derived to calculate GFR expressed in ml/min:

Larsson GFR (Dade Behring) = 77.24 X $(CysC)^{-1.2623}$

Larsson GFR (Dako Cytomation) = 99.43 X $(CysC)^{-1.5837}$

A stronger correlation ($p<0.0001$) was found between CysC and Iohexol clearance ($r^2=0.91$) than between S.Cr and Iohexol clearance ($r^2=0.84$).

3) Hoeck GFR[122]:

This formula was developed on 123 adult patients (including 30 diabetics) with suspected renal disease in Netherlands, comparing the CysC measured by Dade Behring PENIA method (in mg/L) to ^{125}I Iothalamate clearance GFR (reference method).

Hoeck GFR (ml/min/1.73 Sq.m) = [80.35 X (1/CysC)] - 4.32

Bland and Altman analysis showed that the derived formula gave more accurate ($P<0.0001$) and more precise ($P=0.024$) GFR estimates than obtained with the Cockcroft-Gault formula.

4) Filler GFR[123]:

This Formula was developed from 536 children at Ottawa & Berlin (age ranging from 3 months-18 years) comparing CysC measured by Dako PETIA method, to reference GFR measured by ^{51}Cr EDTA clearance.,

log(Filler GFR)=1.962 + [1.123 X log(1/CysC)].

The formula which measured GFR in ml/min/1.73sq.m, had better correlation with EDTA clearance than the Schwartz formula ($r=0.77$ vs. 0.71 respectively).

5) Macissac [124] GFR:

This formula was derived from 251 patients in a Diabetic Clinic in Australia, the iGFR was measured by clearance of ^{99m}Tc-DTPA Clearance (78% having GFR > 60ml/min/1.73sq.m.). The equation is as follows:

Macissac GFR (ml/min/1.73sq.m) = (84.6/ CysC) - 3.2

6) Grubb GFR[125]:

This is by far the most popular of the CysC based eGFR formulae. In this study, data from 536 patients (0.3–93 years), consecutively referred for determination of GFR by plasma Iohexol Clearance, were used for the analysis. CysC was measured using the Dako Cytomation PETIA method. A prediction equation using only CysC (in mg/L) and a prepubertal factor (Pf) assessed GFR (in ml/min/1.73sq.m) equally well or better than the MDRD-2, the Schwartz, and the Counahan–Barratt prediction equations for the juvenile and adult (>18 years) groups. Age did not influence the CysC (Dako) based prediction equation for adults, whereas gender did, but with a factor close to unity (0.948 for females). The equations are:

(1) Grubb age adjusted GFR = 84.69 X ($CysC^{-1.680}$) X Pf

Pf = 1.384 if age < 14yrs; Pf = 1.0 if age ≥ 14yrs.

(2) Grubb age, sex adj. GFR = 87.62 X ($CysC^{-1.693}$) X Pf X S

Pf = 1.376 if age < 14yrs; Pf = 1.0 if age ≥ 14yrs;

S = 0.948 if female; S = 1 if male;

7) Mayo Clinic CysC GFR[126]:

In this study by Rule et.al, CysC (Dade Behring PENIA) and S.Cr levels were obtained from adult patients (n = 460) during an evaluation that included a GFR measurement by Iothalamate clearance (non-radiolabelled Iothalamate measured by capillary electrophoresis). It was notable that, at the same CysC level, GFR was 19% higher in transplant recipients than in patients with native kidney disease. The association between CysC and GFR was stronger among native kidney disease patients than in healthy

persons. As the relationship between CysC and GFR differed across clinical presentations, the GFR equation was modeled using the following variables: CysC (or S.Cr), age, gender, and clinical presentation (CKD patient vs. allograft recipient). The equations are as follows:

(1) Mayo CysC GFR (Tx) = 76.6 X $(CysC)^{-1.16}$

(2) Mayo CysC GFR (CKD) = 66.8 X $(CysC)^{-1.3}$

(3) Mayo S.Cr GFR (CKD) = 273 x $(S.Cr)^{-1.22}$ X $age^{-0.299}$ X 0.738 if female

(4) Mayo Composite GFR(CKD) = $(CysC\ GFR\ X\ S.Cr\ GFR)^{0.5}$

Among transplant recipients, the CysC equation (eqn. (1)) had an $r^2$of 0.768. In CKD patients, the correlation of the CysC equation (eqn. (2), r^2=0.853) was higher than the S.Cr equation derived using the same sample (eqn. (3), r^2=0.827), the MDRD2 equation (r^2=0.825) and the Cockcroft–Gault equation (r^2=0.796).

8) Dade Behring GFR:

Coresh et.al, derived an equation using data from 1,935 adults in the CKD-EPI collaboration consisting of predominantly non-elderly CKD population. The Cystatin C was measured by Dade Behring PENIA method. The GFR equation is as follows:

Dade Behring CysC GFR = 76.7 X $(CysC)^{-1.18}$

9) Zuo Combined GFR[127]:

The Chinese eGFR Investigation Collaboration's study is provides one of the few GFR equations modeled among Asian populations. The GFR equations were based on CysC (Dade Behring PENIA), S.Cr (Jaffe Kinetic method) and reference GFR measured by

^{99m}Tc-DTPA plasma clearance method in a training cohort of 376 randomly selected adult Chinese patients with CKD. Two individual equations, a composite equation of the two and a combined S.Cr - CysC equation that estimate GFR (in ml/min/1.73sq.m) were modeled on this population. They were validated by testing on an additional 191 patients. A final equation was then derived for best fit for all the 567 patients together. The equations are as follows:

(1) Zuo CysC GFR

$$\mathbf{GFR_1 = 86 \times (CysC)^{-1.132}}$$

(2) Zuo S.Cr-CysC GFR

$$\mathbf{GFR_2 = 176 \times S.Cr^{-0.607} \times CysC^{-0.638} \times Age^{-0.171} \quad \times 0.85 \text{ for female}}$$

(3) Modified MDRD2 for Chinese GFR

$$\mathbf{GFR_3 = 175 \times S.Cr^{-1.234} \times Age^{-0.179} \times 0.79 \text{ for female}}$$

(4) Zuo Composite CysC-S.Cr GFR

$$\mathbf{GFR_4 = [(GFR_1) \times (GFR_3)]^{0.5}}$$

(5) <u>Zuo Combined GFR (final)</u>

$$\mathbf{GFR_5 = 169 \times S.Cr^{-0.608} \times CysC^{-0.63} \times Age^{-0.179} \times 0.83 \text{ for female}}$$

The Zuo Combined GFR equation (eqn.5) yielded considerable performance improvement compared with those of S.Cr (eqn.3) or CysC (eqn.1) based equations, and slight superiority to the composite equation (eqn.4) in each stage of CKD, particularly in patients with near-normal kidney function. It gave the best model fit; the R^2 was higher than the individual equations (eqn. 1 & 3), and it also showed the smallest standard error of residual, the smallest relative and absolute difference, and the highest accuracy.

There were several potential limitations to all these studies.

First, patients could have been misclassified into clinical presentations.

Second, among native kidney disease patients, only those with an increased severity of illness, such that a nephrologist would measure the patient's GFR, may have been represented. Thus, patients with microalbuminuria and a normal serum creatinine level could have been inadequately represented.

Third, the generalizability of the CysC equations needs to be tested in other centers with more diverse racial groups and different mixtures of CKD etiologies.

Finally, any calibration differences between the various methods used for assay of CysC used in these studies and other CysC assays can lead to inaccurate GFR estimates.

In summary, it appears that in certain populations, CysC may be more accurate for assessment of kidney function than S.Cr. Whether measurement of CysC levels will improve patient care is at present unknown.

4.0 STATISTICAL METHODS FOR GFR FORMULAE COMPARISON

Review of the literature showed great heterogeneity in how the performance of prediction equations was assessed. Improper techniques and inadequate analyses have marred the clinical value of most of the studies in GFR estimation. Hence it is important to review the basic statistical analyses required while studying GFR prediction equations and understand the shortcomings of the same.

4.1 Bias, Precision and Accuracy

In choosing a prediction equation to estimate GFR, one should consider both the bias and precision of the equation-generated estimates. **Bias** expresses the systematic deviation from the gold standard measure of GFR. A prediction equation that consistently overestimates or underestimates the gold standard measure of GFR yields a biased estimate. The mean difference between the actual measured GFR (gold standard) and the estimated GFR based on an equation is a valid measure of **bias**. The median difference provides a measure that is valid and less susceptible to influence by outliers. **Precision** expresses the variability (or dispersion) of prediction equation estimates around the gold standard GFR measure. The standard deviation of the difference between the measured and estimated GFR is a measure of **precision**. The difference from the gold standard can also be expressed as a **relative difference**, i.e., percent difference from the measured GFR. This has the advantage of allowing for the decreased absolute precision in estimating higher values of GFR. Clinically this is relevant, as there is less concern about the difference between 100 and 130 mL/min/1.73 Sq.m than between 30 and 60 mL/min/1.73 Sq.m. **Accuracy** combines precision and bias. A useful measure of accuracy is a description of percentiles of the distribution of the differences between estimated and measured GFR. In other words, if 99% of the time a prediction equation yields an estimate within 10% of the measured GFR, it would be a very accurate and useful clinical tool. Achieving a high level of accuracy requires both low bias and high precision. Description of the percent of estimates falling within 30% and 50% above or below the measured GFR is a useful measure of accuracy.

4.2 Analysis and Interpretation of Data

Correlation coefficients are frequently cited in the literature on prediction equations. However, they are inadequate for measuring the validity of a method in estimating GFR for two reasons.

Although correlation coefficients (r) measure the association between prediction equation and measured GFR, the correlation coefficient is highly dependent on the distribution of GFRs in the study population selected. Even poor estimates can discriminate between a GFR of 20 and 120 very reliably.

Second, correlation measures ignore bias and measure relative rather than absolute agreement. For example, in the MDRD Study the Cockcroft-Gault equation had a similar correlation to GFR as the MDRD Study equation but overestimated GFR by 19%[4]. Analogous studies in children show similar limitations in assessing the utility of a prediction equation by virtue of its correlation coefficient [6]. The correlation between inulin clearance and estimated GFR by the Schwartz formula was 0.905, while in the same study, the standard deviation of the difference between the reference value (C_{in}) on the predicted value was 28.6%, indicating limited precision.

Regression equations are another commonly used measure of prediction equations. Regression equations relating an estimate of GFR and the measured GFR provide an estimate of systematic bias, in the relationship between the two variables, as well as the correlation and residual root mean error, measures of precision. However, such regression analyses have two drawbacks.

First, ordinary least square regression does not allow for measurement error in the X-variable. As a result, the regression equation provides a prediction equation conditional of

the X-value rather than an unbiased estimate of the relationship. For example, a regression of one GFR measure on a second GFR measure, using the same technique on another day, would have a slope that is substantially lower than 1.0 and an intercept greater than zero. The importance of measurement error in the X-values depends on the correlation, which in turn depends on the study population.

Second, regression equations cannot be pooled across different studies.

Finally, evaluation of the accuracy of any equation for estimating GFR must be made in an independent group from the group in which the equation itself was derived.

5.0 GFR ESTIMATION IN INDIA

Until now, a normal reference range for GFR in healthy adult Indians (usually evaluated in potential kidney donors) has not been determined and values from western population are being used as reference. Barai S et al[128] , on studying 610 Indian patients' (250 males, 360 females, average age 35.16 years) GFR by ^{99m}Tc-DTPA plasma clearance by Russell's two sample method, observed a mean GFR of 82.3 ± 21.3 and 80.8 ± 18.1 ml/min/1.73Sq.m in adult males and females were respectively. Similar reports have been documented other studies[129]. Such a low average GFR in healthy Indian adults is a subject of debate .We need better methods to confirm the reference GFR for our population. In spite of the various shortcomings, DTPA clearance or scintigraphy and the estimation equations (unadjusted for Indian subjects) have been utilized in these studies. In India, monetary limitations and lack of standardization of assays precludes accurate

assessment of GFR. For us, the question of a standard test for GFR measurement remains unanswered.

Studies have been performed to look at the applicability of estimations using creatinine based equations. Mahajan et al[130] studied the performance characteristics of the Cockcroft Gault formula, MDRD1, MDRD2, and the 24 hour urine creatinine clearance among 173 voluntary kidney donors (mean age 44.1 yrs, M:F = 1:3) comparing with GFR assessment by ^{99m}TcDTPA clearance. However, there was very poor correlation of all the formulae with the DTPA clearance, implying that these equations are suboptimal for donor evaluation. Cystatin C has not been well studied in the Indian population. John GT et al[131], concluded that because of its large intraindividual variations, serial serum CysC estimation was very poor in detecting reduced renal function. There are no published studies in Indexed journals that looked at the applicability of the various eGFR prediction formulae and the CysC in the Indian renal allograft recipient population.

AIM

To study the agreement between GFR estimated by various prediction formulae based on Serum Creatinine and Cystatin C with the GFR measured by ^{99m}Tc DTPA renal scintigraphy among renal allograft recipients.

To determine which estimated GFR prediction formula predicts GFR measured by ^{99m}Tc DTPA renal scintigraphy with precision and accuracy in renal allograft recipients.

PATIENTS AND METHODS

DESIGN AND LOCATION: This prospective study was conducted at the Department of Nephrology, Christian Medical College, Vellore.

PATIENTS: Renal allograft recipients who satisfied the following Inclusion and exclusion criteria were included:

INCLUSION CRITERIA: Renal allograft recipients

Renal transplantation done at Christian Medical College, Vellore

Completed six months of intensive follow up

Stable graft function

On Prednisolone dose of 7.5 – 12.5 mg/day

EXCLUSION CRITERIA: Incomplete follow up

Unstable graft functions or Graft failure

DURATION: Patients who underwent renal transplantation from January 2006 to June 2007 were included and followed up to January 2008.

METHODOLOGY: Renal allograft recipients who underwent renal transplantation at CMC, Vellore, were followed up for first six months intensively. As per existing protocols, at completion of the 6 months of intensive follow-up, patients underwent ^{99m}Tc-DTPA scintigraphy to assess graft function. Anthropometric measurements of height and weight were measured. Biochemical analysis such as serum creatinine, serum

urea, serum albumin and serum Cystatin C were performed at this point. GFR estimated from various prediction equations based on serum creatinine and Cystatin C were compared with the GFR obtained from DTPA clearance study.

MEASUREMENTS:

1) Anthropometry: Height and Weight were measured on height and weighing scales, standardized according to the rules of Department of Legal Metrology of the Government of India. Anthropometric calculations were based on the following formulae:

BSA = Body surface area in Sq.m. (Du Bois [9] formula)

$= 0.20247 \text{ X } (\text{Height in cms}/100)^{0.725} \text{ X } (\text{Weight in Kg})^{0.425}$

LBW = Lean Body Weight[132] in Kg

$= [(1.10 - (0.03 \text{ X sex})] \text{ X } (\text{weight})) - \{[128 + (\text{sex X } 20)] \text{ X } [(\text{weight}/\text{height})^2]\}$

IBW = Ideal Body Weight[133, 134]

$= 50 + \{2.3 \text{ X } [(\text{height} - 152.4) / 2.54]\}$ for males

$= 49 + \{1.7 \text{ X } [(\text{height} - 152.4) / 2.54]\}$ for females

2) Clinical Biochemistry:

Serum Creatinine: Serum creatinine was measured (in mg/dl) by the Modified Jaffe's Kinetic Alkaline Picrate method (colorimetry) without deproteinization using Automated Chemistry Analyzer Olympus AU 2700 (Japan).

Principle: Creatinine + Picric acid ----------> Creatinine picramate

Explanation: Creatinine in the serum forms a coloured complex with picrate in alkaline solution. The rate of absorbance change of the coloured complex is proportional to the creatinine concentration.

Serum Urea: Serum urea is measured (in mg/dl) by Berthelot's Method (enzymatic colorimetric) using the Automated Chemistry Analyzer Olympus AU 2700 (Japan).

Principle: **The Berthelot reaction, is a reaction where ammonia reacts with hypochlorite, phenol, a catalyst, and alkali to produce a stable blue complex (indophenol).**

Explanation: **Urea measurement in our lab is based upon a modified Berthelot reaction wherein urease hydrolyzes urea to ammonia and carbamic acid. Carbamic acid**

spontaneously decomposes into ammonia and carbon dioxide. Ammonia reacts with salicylate, nitroferricyanide and an alkaline solution of hypochlorite to yield a blue-green chromophore which is measured photometrically and is proportional to the amount of urea in the sample.

Serum Albumin: Serum Albumin was measured (in gm/dl) by colorimetric, end point bromocresol green (BCG) method using the Automated Chemistry Analyzer Olympus AU 2700 (Japan).

Principle: BCG + Albumin----------→Complex with color change

Explanation: BCG is a yellow indicator, which changes color from yellow to blue green when binding to albumin. Turbidity is avoided by addition of Brij- 35

Serum Cystatin C: Serum Cystatin C was measured (in mg/L) by Particle Enhanced Nephelometric Immuno Assay (PENIA) by Dade Behring BN 100 Nephelometer.

Principle and Explanation: Polystyrene coated with specific antibodies to human Cystatin C aggregates when mixed with particles containing Cystatin C. These aggregates scatter a beam of light passing through the sample. The intensity of the scattered light is proportional to the concentration of the Cystatin C in the sample. The result is evaluated by comparison with a standard of known concentration.

3) Nuclear Medicine: Measured GFR (mGFR) by ^{99m}Tc-DTPA Renal scintigraphy:

^{99m}Tc DTPA scintigraphy performed on the patients after the administration of 130 MBq of the radioactive isotope and the scans were performed based at specified time intervals. The gamma camera based clearance methods are based on detection of the percent dose uptake of 99mTc-DTPA during the time interval in which none of the 99mTc-DTPA has entered the renal pelvis. Thus all filtered and secreted 99mTc-DTPA is within the kidney. DTPA clearance is estimated using the Modified Gates[31] method as follows:

$$\textbf{DTPA Clearance} = [9.8127 \times (\text{Graft uptake \%}) - 6.82519] \times 1.73 / \text{BSA}$$

$$\textbf{Uptake \%} = 100 \times \text{Integral interval} / (\text{Injected dose} \times \text{DCA})$$

$$= \frac{[(\text{Graft Counts} - \text{Background counts}) / \text{DCA}]}{\text{Pre syringe counts} - \text{post syringe counts}} \times 100$$

DCA = exp (-0.153 X GD),

GD = graft depth in cm (Measured by an on-site CT scan / USG From skin to the mid point of the mid polar renal tissue)

Injected dose is determined by:

- acquisition of a full and empty syringe,
- entering the injected dose and time of measurement of injected dose.

Integral Interval = 120 -180 seconds

The DTPA Clearance is read as measured GFR (mGFR) adjusted to a BSA of 1.73Sq.m.

4**) Estimation of GFR (eGFR formulae):**

The following GFR formulae were used for estimation of GFR (pre corrected to report GFR in ml/min/1.73Sq.m.):

1. **<u>eGFR based on serum creatinine:</u>**

*1.**Cockcroft-Gault GFR***

CGGFR = {[(140-age) X weight] / (72 X S.Cr)} X (0.85 if female) X 1.73/BSA

CGIWGFR = {[(140-age) X IBW] / (72 X S.Cr)} X (0.85 if female) X 1.73/BSA

CGLWGFR = {[(140-age) X LBW] / (72 X S.Cr)} X (0.85 if female) X 1.73/BSA

*2. **Six variable Original MDRD1 GFR***

MDRD-1 GFR = 170 X $(S.Cr)^{-0.999}$ X $age^{-0.176}$ X $(Ur \times 2.78)^{-0.17}$ X $alb^{0.318}$ X (0.762 if female) X (1.18 if African-American)

*3.**Four variable abbreviated MDRD2 GFR***

MDRD2 GFR = 186 X $(S.Cr)^{-1.154}$ X $(Age)^{-0.203}$ X (0.742 if female)

X (1.210 if African American)

4.***Nankivell GFR***

NKVLGFR = [6700 / (S.Cr X 88.4)] + (weight/4) - (urea/12) – [100/ (height) ^{2}] + 35

– [10 (if female)]

5.***Mayo clinic quadratic GFR***

MCQGFR = exp [1.911+ (5.249 / S.Cr) – (2.114 / S.Cr^2) - (0.00686 X Age)

– 0.205(if female)]

2. **<u>eGFR based on serum Cystatin C:</u>**

6.***Le Bricon GFR:*** **LEBGFR** = 78 X (1/CysC) + 4

7.***Larsson GFR:*** **LSNGFR** = 77.24 X (CysC)$^{-1.2623}$ X 1.73/BSA

8.***Hoeck GFR:*** **HKGFR** = [80.35 X (1/CysC)] - 4.32

9.***Grubb age adjusted GFR:*** **GAGFR** = 84.69 X (CysC$^{-1.680}$) X Pf

Pf = 1.384 if age < 14yrs; Pf = 1.0 if age ≥ 14yrs.

10. ***Grubb age & sex adjusted GFR:*** **GASGFR** = 87.62 X (CysC$^{-1.693}$) X Pf X S

Pf = 1.376 if age < 14yrs; Pf = 1.0 if age ≥ 14yrs; S = 0.948 if female; S = 1 if male;

11. ***Macissac GFR:*** **MACGFR** = (84.6/ CysC) - 3.2

12. ***Mayo Clinic CysC Tx GFR:*** **MCTXGFR** = 76.6 X (CysC)$^{-1.16}$

13. ***Zuo Combined GFR:***

ZCOMGFR = 169 X S.Cr$^{-0.608}$ X CysC$^{-0.63}$ X Age$^{-0.179}$ X 0.83 for female

The eGFRs were pre-adjusted to a BSA of 1.73 Sq.m for comparability.

SAMPLE SIZE:

<u>Parameter</u>	***<u>Value</u>***
Sample reliability value	0.52
Population reliability value	0.95

Study sample size	134
Alpha error	5
Power obtained	99.9%

STATISTICAL ANALYSIS:

Statistical analysis was performed using the SPSS version 11.5 and Microsoft Excel 2003. The various analytical techniques used for comparison of the estimated and measured GFR are as follows:

1. **Measures of central tendency and distribution** – Mean ± standard deviations were used for normally distributed data and median & range (min – max) was used for skewed data to avoid the outlier effect.
2. An **intra-class correlation coefficient** (one way random) was calculated for the eGFR and the mGFR and their significance was determined. Scatter plots were used for graphical representation of the same.
3. Bias and precision were calculated based on the following definitions and graphically demonstrated using **Bland -Altman** analysis plots
    a. **Bias** = Mean difference (eGFR – mGFR)
    b. **Precision** – 2 Std. deviation of Bias = 2 X S.D (eGFR – mGFR)
4. Relative Bias and Accuracy of eGFRs to predict mGFR were calculated:
    a. **Relative Bias** - % of difference between eGFR & mGFR (as % of mGFR)

        Relative Bias (RB) = (Bias / mGFR) X 100

    b. **Accuracy** – % of eGFR values falling within a set RB : Accuracy @
        i. 30% = [(no. of eGFR values with RB ≤ 30%) / (total =134)] X 100

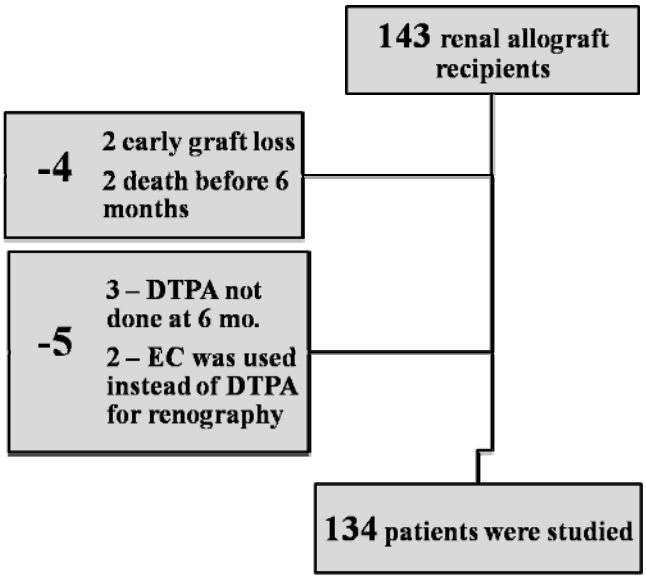
ii. 50% = [(no. of eGFR values with RB ≤ 50%) / (total =134)] X 100

5. **Kappa statistics** for agreement for GFR based classification (into three groups, GFR<30, 30-60 and >60 ml/min/1.73Sq.m) by the various eGFR equations and the mGFR were studied. The agreement was ascertained by the following scale: <0.20 - Poor; 0.21-0.40 - Fair; 0.41-0.60 - Moderate; 0.61-0.80 - Good; 0.81-0.99 -Very good; 1.00 – Perfect agreement. The percentage of total number of patients misclassified into different group by the eGFR equation in study was also derived from the cross-tabulations.

RESULTS

Patient Profile: During the enrollment period between January 2006 to June 2007, 143 patients underwent renal transplantation at Christian Medical College, Vellore. Of them, 134 patients were eligible and were included into the study:

Figure 7.Case profile of the study



Clinical Details: The study comprised predominantly of adults. Seven patients had age between 15 and 18 years. Majority of them received their grafts from live related donors

Table 7. Demographic profile

Total Number of patients	134
Males : Females	101:33 (3:1)
Age (in years) mean ± S.D	34.0 ± 11.5
Age Median (min-max)	33.0 (15.0 – 61.0)
Live – related renal transplantation	128 (95.5%)
Deceased Donor renal transplantation	6 (4.5%)

Nearly half the patients received the standard Prednisolone, Tacrolimus and Mycophenolate based triple immunosuppression. Another 40% received Cyclosporine.

Table 8. Immunosuppression

Immunosuppression	*Number*	*Percentage (n=134)*
Pred + CsA + Aza	31	23.1
Pred + CsA + MPA	25	18.7
Pred + Tac + Aza	10	7.5
Pred + Tac + MPA	65	48.5
Pred + Siro/Eve + MPA / Aza	3	2.2

Anthropometry: The Anthropometric details were as follows. The average height and weight were 164 cm and 60Kg. However, there were some outliers. The mean BSA in our population was 0.07Sq.m less than the standard 1.73Sq.m in the west.

Table 9. Anthropometric profile

Measure	*mean ± S.D*	*Median (min- max)*
Height in cm	163.7 ± 8.6	165.0 (148.0 - 185.0)
Weight in Kg	59.7 ± 9.9	60.0 (42.0 – 124.0)
Body surface area in Sq.m.	1.64 ± 0.15	1.66 (1.33 – 2.39)
Lean Body weight in Kg	47.3 ± 6.7	47.1 (33.3 – 74.3)
Ideal Body weight in Kg	59.8 ± 8.1	61.4 (46.0 – 79.5)

Clinical Chemistry: The median serum Creatinine was 1.2 mg/dl, reflecting general well being of the graft at the end of six months follow up. This was substantiated by the

fact that majority of the patients have a 24 hour urine protein less than 1 gm/day. The serum Biochemical profile of our patients in given in Table 10.

Table 10. Biochemical profile

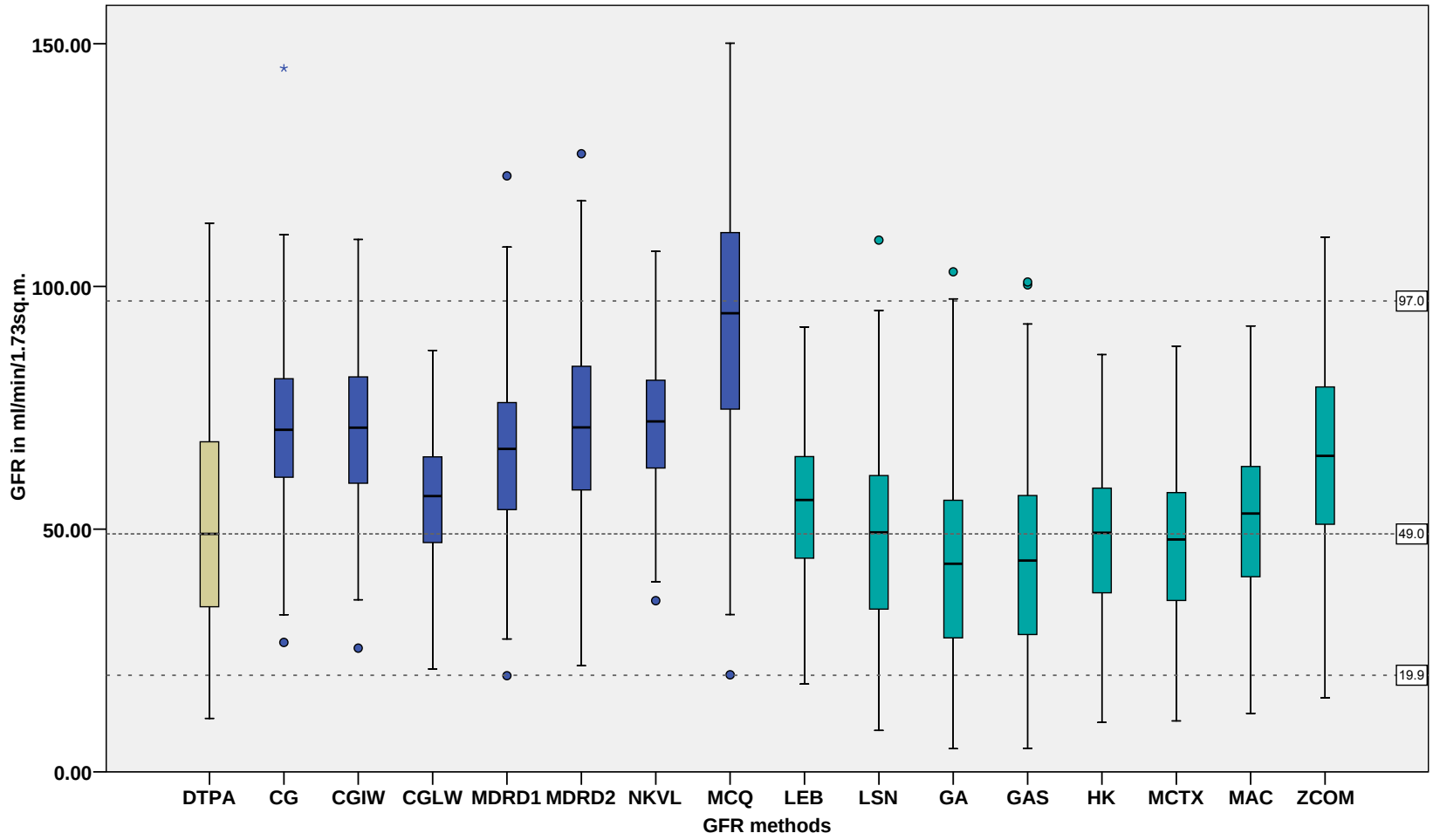
Measure	***mean ± S.D***	***Median (min- max)***
Serum Creatinine in mg/dl	1.3 ± 0.4	1.2 (0.7 – 3.2)
Serum Urea in mg/dl	33.8 ± 14.2	31.0 (15.0 – 110.0)
Serum Albumin in gm/dl	4.2 ± 0.4	4.3 (2.6 – 5.1)
Serum Cystatin C in mg/L	1.67 ± 0.63	1.50 (0.89 – 5.55)
24 hour urine protein in mg/day	245.8 ± 377.7	150.0 (48.0 – 3100.0)

Measured and Estimated GFR: The median, 5^{th} and 95^{th} percentiles of mGFR from DTPA renal scintigraphy were 49, 19.9 and 97 ml/min/1.73Sq.m respectively. The distribution of the various eGFRs are depicting in Table 11 and Figure 8. The eGFR ranges for the S.Cr. based equations is much higher compared to the mGFR. However, the Cockcroft-Gault equation using lean body weight instead of actual body weight has a distribution closer to the mGFR range. Cystatin C based equations predicted eGFR ranges are similar (if not lower) compared to the mGFR distribution. The equation with the a median eGFR similar to the mGFR range would be the Larsson and the Macissac CysC based equation.

Table 11. The mGFR and eGFR distribution

Measure (in ml/min/1.73Sq.m.)	*mean ± S.D*	*Median (min- max)*
99mTc-DTPA Clearance (mGFR)	52.1 ± 22.6	49.0 (11.0 – 113.0)
eGFR based on S.Creatinine:		
CGGFR	70.7 ± 16.5	70.5 (26.7 – 145.0)
CGIWGFR	71.2 ± 16.9	70.9 (25.5 – 109.7)
CGLWGFR	56.1 ± 12.3	56.8 (21.2 – 86.8)
MDRD1GFR	65.4 ± 17.5	66.5 (19.8 – 122.8)
MDRD2GFR	71.3 ± 18.3	70.9 (21.9 – 127.3)
NKVLGFR	71.8 ± 14.0	72.2 (35.3 – 107.2)
MCQGFR	92.2 ± 25.6	94.5 (20.0 – 150.1)
Measure (in ml/min/1.73Sq.m.)	*mean ± S.D*	*Median (min- max)*
eGFR based on S. Cystatin C:		
LEBGFR	55.4 ± 14.4	56.0 (18.1 – 91.6)
LSNGFR	49.6 ± 19.4	49.3 (8.5 – 109.5)
GAGFR	43.9 ± 19.9	42.9 (4.8 – 103.0)
GASGFR	44.4 ± 19.9	43.5 (4.8 – 100.9)
HKGFR	48.6 ± 14.9	49.2 (10.2 – 86.0)
MCTXGFR	47.6 ± 15.3	47.9 (10.5 – 87.7)
MACGFR	52.5 ± 15.7	53.2 (12.1 – 91.9)
ZCOMGFR	65.4 ± 18.8	65.1 (15.3 – 110.2)

Figure 8. Distribution of the mGFR and various eGFR values. Dotted lines represent the median, 5th and the 95th percentile of mGFR values



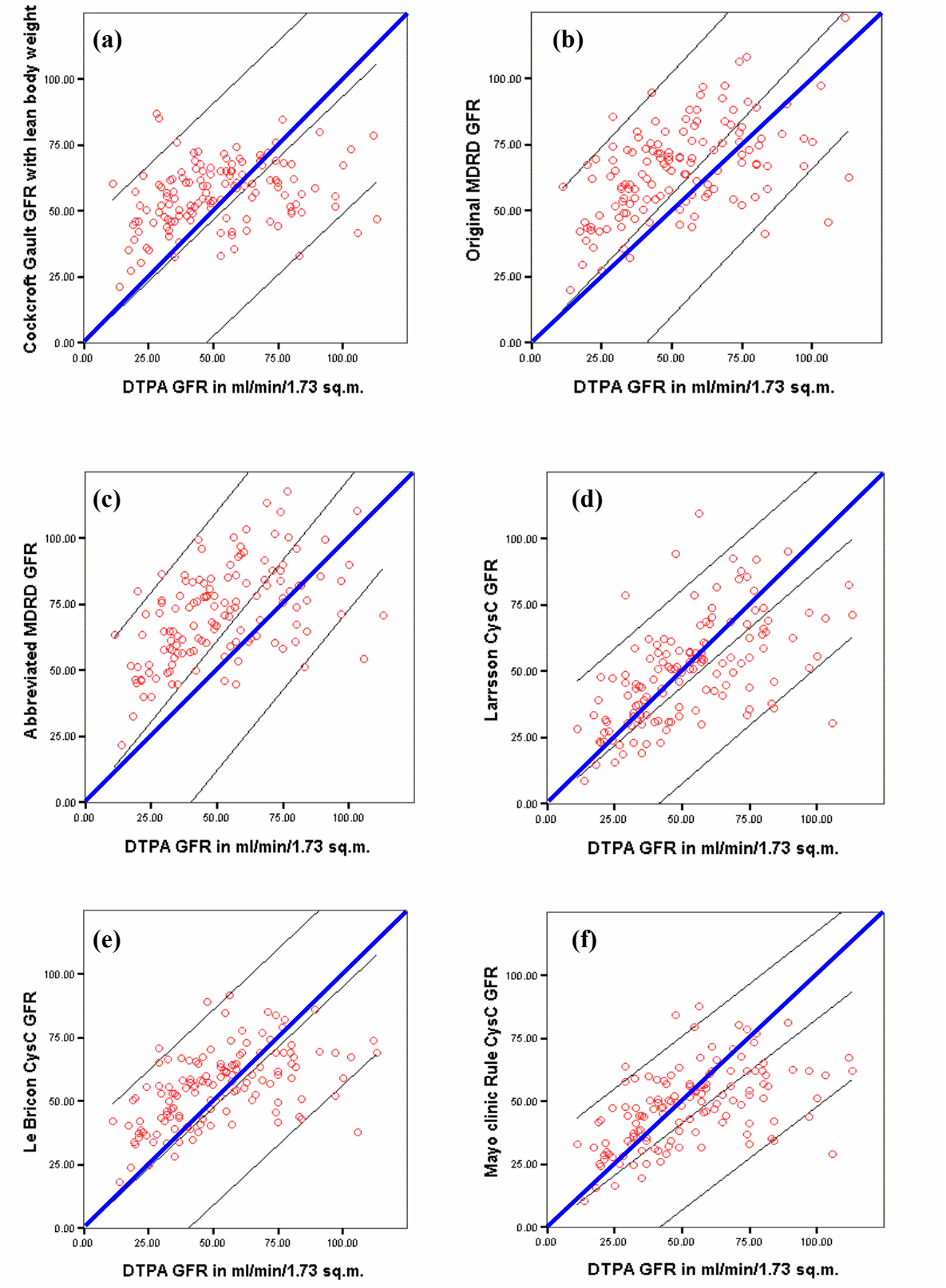
Comparison between the mGFR and eGFR:

1. Intra Class Correlation Coefficients: The various intra-class correlation coefficients for the various eGFR equations are listed in Table 12. Most of the S.Cr based equations have poor or no correlation with mGFR. The commonly used Cockcroft Gault GFR and the Nankivell GFR (that was developed exclusively from renal transplant recipients) fared the worst with ICC of 0.03 and 0.05 respectively. However, among them the formulae with higher degree of correlation are the original six variable MDRD equation (MDRD1 with ICC=0.36), closely followed by the Abbreviated four variable MDRD equation (MDRD2 with ICC = 0.26) and the Cockcroft-Gault GFR using lean body weight (CGLW with ICC = 0.25). In contrast, the CysC based equations fared better. The best correlating eGFR equations were the Larsson eGFR and the Macissac eGFR (ICCs of 0.57 and 0.50 respectively). The widely used Grubb CysC equations had an ICC of 0.46. Mayo Clinic CysC GFR and Le Bricon GFR (both derived from renal transplant recipients) had ICC of 0.47 each. None of the equations attained an ICC > 0.60.

Table 12. Intra Class Correlation between various eGFRs and the mGFR

Variable	*ICC*	*95% C.I.*		*p value*
		Lower bound	*Upper bound*	
CGGFR	0.03	-0.14	0.19	0.371
CGIWGFR	0.12	-0.05	0.28	0.088
CGLWGFR	0.25	0.08	0.40	0.002
MDRD1GFR	0.36	0.20	0.49	<0.001
MDRD2GFR	0.26	0.09	0.41	0.001
NKVLGFR	0.05	-0.12	0.22	0.269
MCQGFR	-0.06	-0.23	0.11	0.756
LEBGFR	0.47	0.33	0.60	<0.001
LSNGFR	0.57	0.45	0.68	<0.001
GAGFR	0.46	0.32	0.58	<0.001
GASGFR	0.46	0.32	0.58	<0.001
HKGFR	0.48	0.34	0.60	<0.001
MCTXGFR	0.47	0.33	0.60	<0.001
MACGFR	0.50	0.36	0.62	<0.001
ZCOMGFR	0.42	0.27	0.55	<0.001

Figure 9: Scatter plots for eGFRs vs. mGFR



2) Bland-Altman Analysis of Bias and Precision:

The S.Cr based eGFR equations seem to overestimate the GFR by an average of 15-20ml/min/1.73Sq.m. Least bias was observed for the Cockcroft-Gault formula using the lean body mass for calculation (CGLW with Bias of +4.0 ± 22.2ml/min/1.73sq.m.). However, the CysC based equations had comparatively much less bias (1-10 ml/min/1.73Sq.m) Least bias was observed for the Larsson GFR (-2.4 ± 19.5 ml/min/1.73Sq.m) and Macissac GFR(0.5 ± 19.5 ml/min/1.73Sq.m) . Precision (2 SD of the Bias) was equally poor for all the eGFR equations (approx. ±40ml/min/1.73sq.m.). The precision was marginally better for the CysC based equations (±35-40ml/min/1.73Sq.m) compared to the S.Cr based equations (±40-50ml/min/1.73Sq.m).

3) Relative Bias and Accuracy:

Relative Bias was much higher for the S.Cr based equations (40-50%) compared to the CysC based equations (24-28%). Accuracy at 30% and 50% was poor for all S.Cr based equations (30-40% and 50-60% respectively). Among the S.Cr based eGFRs, Cockcroft-Gault GFR using lean body weight had the least 'relative' bias (32.1%) and best accuracy (@30% - 47.8%, @50% - 72.1%). Overall least relative bias and best accuracy was observed for the Larsson , Macissac and the Mayo Clinic CysC GFRs.

Table 13. Bias and Precision of eGFR to mGFR

eGFR	*Bias (mean±SD) ml/min/1.73m²*	*95% C.I. of bias ml/min/1.73m²*		*Precision ± 2SD ml/min/1.73m²*	*p Value*
		Lower bound	*Upper bound*		
CGGFR	18.7± 24.2	14.5	22.8	48.3	<0.001
CGIWGFR	19.2 ± 22.4	15.3	23.0	44.7	<0.001
CGLWGFR	**4.0 ± 22.2**	**0.2**	**7.8**	**44.3**	**0.039**
MDRD1GFR	13.4 ± 20.2	9.9	16.8	40.4	<0.001
MDRD2GFR	19.2 ± 20.0	15.8	22.6	40.0	<0.001
NKVLGFR	19.7 ± 21.6	16.0	23.4	43.2	<0.001
MCQGFR	40.1 ± 21.9	36.3	43.8	43.9	<0.001
LEBGFR	3.3 ± 19.3	0.0	6.6	38.6	0.049
LSNGFR	**-2.4 ± 19.5**	**-5.8**	**0.9**	**38.9**	**0.149**
GAGFR	-8.2 ± 21.0	-11.8	-4.6	42.1	<0.001
GASGFR	-7.7 ± 21.7	-11.3	-4.1	42.3	<0.001
HKGFR	-3.5 ± 19.8	-6.8	-0.2	38.7	0.040
MCTXGFR	-4.5 ± 19.5	-7.8	-1.2	38.9	0.008
MACGFR	**0.5 ± 19.5**	**-2.9**	**3.8**	**39.0**	**0.784**
ZCOMGFR	13.4 ± 19.3	10.1	16.7	38.6	<0.001

Figure 10.Bland Altman Plots for eGFR – mGFR pairs

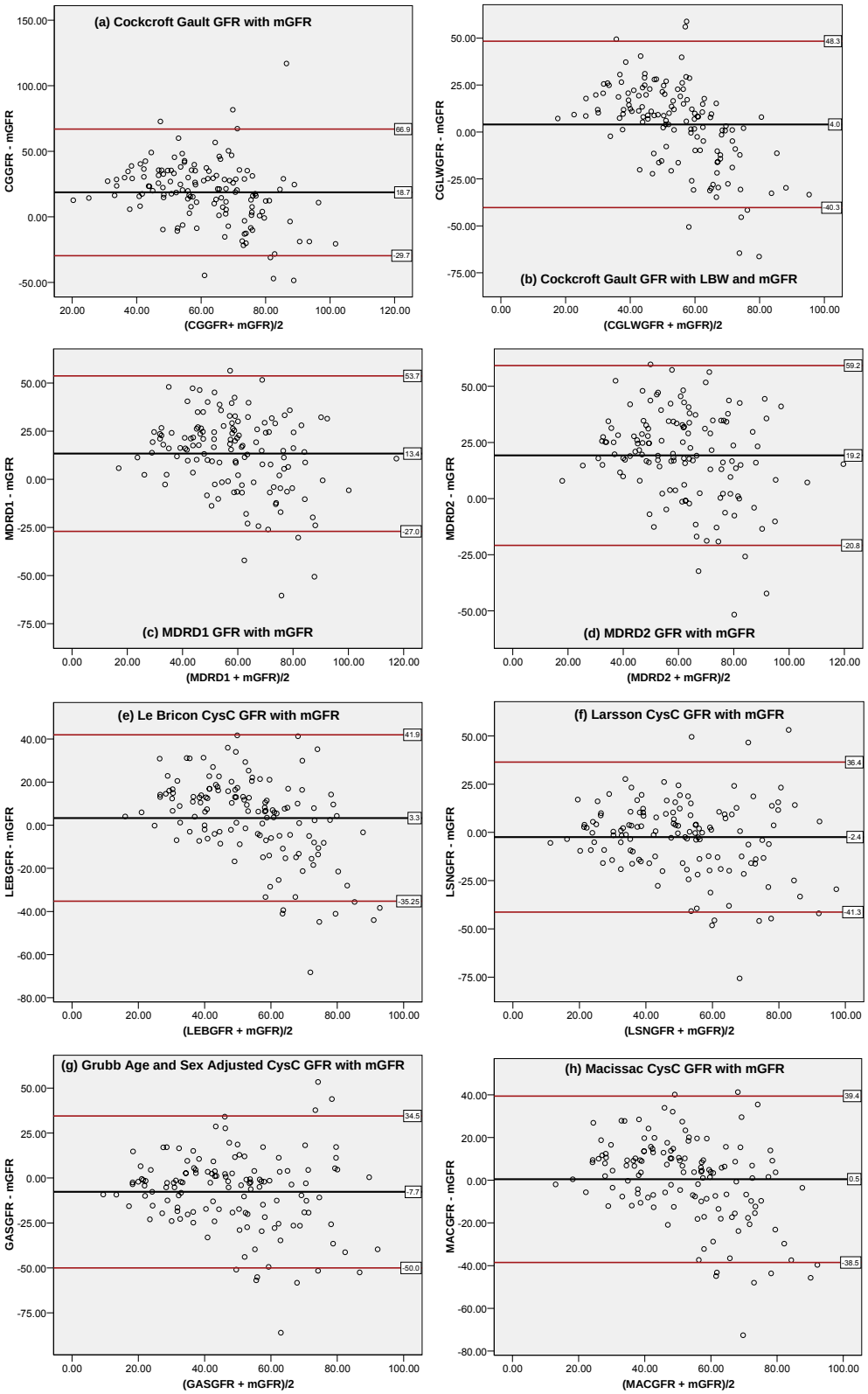


Table 14. Relative Bias and accuracy of eGFR to mGFR

eGFR Equation Tested	*Median Relative Bias (RB) (%)*	*Accuracy at (%)*	
		30% RB	*50% RB*
CGGFR	45.9	35.8	52.2
CGIWGFR	51.1	31.3	50.0
CGLWGFR	**32.1**	**47.8**	**72.1**
MDRD1GFR	41.3	38.1	59.7
MDRD2GFR	52.1	32.1	48.5
NKVLGFR	48.2	33.6	50.7
MCQGFR	81.0	12.7	29.1
LEBGFR	26.7	55.2	76.9
LSNGFR	**23.7**	**61.2**	**87.3**
GAGFR	**28.1**	**61.2**	**81.3**
GASGFR	**26.7**	**62.7**	**80.6**
HKGFR	24.8	58.2	85.8
MCTXGFR	**24.8**	**61.9**	**85.8**
MACGFR	24.6	59.7	83.6
ZCOMGFR	37.7	40.3	62.7

3) Agreement statistics on GFR classification: GFR was classified into three groups - <30, 30-60 and >60 ml/min/1.73m^2 based on mGFR and the various eGFR. The Kappa statistics of agreement between the eGFR and the mGFR classes were studied. The S.Cr based equations misclassified nearly 50-60% of the patients to a different GFR group.

They consequently had the worst agreement uniformly (K= 0.05-0.20). CysC based equations fared slightly better with % misclassified being 30-40% (K = 0.30-0.45). The equations that best agreed with mGFR were Larsson and the Mayo Clinic CysC GFR.

Table 15. Kappa statistics for GFR classification based on mGFR and various eGFRs

GFR	***GFR class (no. of patients)***			***%misclassified***	***Kappa***	***Agreement***	***p value***
	≥60	***30-60***	***<30***	***n=134***	***statistics***		
DTPAGFR	44	69	21	-	-	-	-
CGGFR	102	31	1	56.7	0.10	Poor	0.058
CGIWGFR	99	34	1	59.0	0.06	Poor	0.290
CGLWGFR	54	78	2	50.8	0.10	Poor	0.130
MDRD1GFR	79	52	3	49.3	0.18	Poor	0.004
MDRD2GFR	98	35	1	53.8	0.14	Poor	0.011
NKVLGFR	107	27	0	59.7	0.07	Poor	0.128
MCQGFR	116	17	1	62.0	0.05	Poor	0.255
LEBGFR	51	79	4	41.8	0.26	Fair	<0.001
LSNGFR	**36**	**78**	**20**	**34.2**	**0.42**	**Moderate**	<0.001
GAGFR	26	69	39	40.3	0.36	Fair	<0.001
GASGFR	28	69	37	39.5	0.37	Fair	<0.001
HKGFR	30	91	13	33.6	0.40	Fair	<0.001
MCTXGFR	**29**	**87**	**18**	**31.3**	**0.45**	**Moderate**	<0.001
MACGFR	44	81	9	40.2	0.29	Fair	<0.001
ZCOMGFR	76	55	3	46.3	0.23	Fair	<0.001

DISCUSSION

Estimation of the glomerular filtration rate in is an important part of management of renal transplant recipients. As direct measurements of GFR are cumbersome, several prediction equations have been derived on various population subsets to predict the GFR based on endogenous serum markers. Nephrologists rely on direct methods only when the clinical picture does not correlate with the eGFR. Several studies have evaluated the utility of the eGFR equations in the renal allograft recipients in the west, though none have been tested or validated among the Brown Caucasian allograft recipients of the Indian subcontinent. Our study was designed to assess the performance characteristics of the various S.Cr and CysC based eGFR equations in predicting the measured GFR (by 99mTc DTPA renography) among renal allograft recipients of the Indian subcontinent.

Our results show that the mGFR tends to differ significantly from the eGFR. The S.Cr based equations fared poorly in all respects of comparison with mGFR. Their eGFR ranges were much higher than the mGFR range. They had higher bias, poor precision, low accuracy and poor agreement with mGFR. Nearly 60% of patients are misclassified into different GFR groups using S.Cr eGFRs . The widely used Cockcroft Gault GFR had one of the worst performance characteristics. The abbreviated four variable MDRD2 equation was not better than the other S.Cr eGFRs. The Nankivell GFR was derived on a cohort of Australian renal allograft recipients to estimate GFR measured by DTPA clearance. In spite of the similarities in test situation to our study, it had poor correlation, high bias and low accuracy compared to other S.Cr eGFRs. Only the original six variable MDRD1 and the Cockcroft-Gault equation using lean body weight fared slightly better than the rest. Nevertheless, their performance characteristics were generally poor.

Comparatively, the CysC based equations have fared better. They had lesser bias, though precision was similar to the S.Cr based equations. They also had greater accuracy, and better agreement with mGFR. Among the various CysC based equations the Larsson GFR, the Macissac GFR and the Mayo Clinic CysC GFR estimates have the best performance characteristics. The Zuo combined GFR based on both S.Cr and CysC derived from Chinese population, performed worse than all CysC based equations, possibly due to S.Cr related factors.

There are several important lessons from these results. S.Cr seems to be a poor indicator of renal function in renal allograft recipients. In most of the cases, S.Cr based eGFR overestimates renal function. The MDRD2 and Cockcroft Gault equations, recommended by NKF-KDOQI may not apply to renal allograft recipients of Indian subcontinent. As S.Cr is widely and frequently used for monitoring renal allograft recipients, it is important to bear in mind that early reductions in GFR may be underestimated by the S.Cr based eGFR equations. Care of the renal allograft recipient in the early post transplant period can be compromised by such an overestimated view of his/her GFR.

There are many reasons for these observations. Tubular secretion is increased in renal allografts, resulting in lowering of S.Cr without an increment in GFR. The degree of tubular secretion is not uniform and seems independent of the GFR. In addition, hyperfiltration by renal allografts may also reduce S.Cr. The malnourished chronic kidney disease patient with poor muscle mass has significant weight gain in the first 6 months after renal transplantation. However, most of the weight gain is due to body fat and not muscle mass. Hence, S.Cr levels may be low for the given body weight, resulting in overestimation of GFR. The fact that Cockcroft Gault GFR using Lean Body Weight

had better performance characteristics compared to all other S.Cr eGFRs supports the hypothesis. The S.Cr. in our study, measured by Jaffe reaction (alkaline picrate) using an auto-analyzer, has not been standardized to the IDMS traceable S.Cr values. This is especially of importance while using the abbreviated four variable MDRD2 equation. Even though we have used the equation meant for the non standardized creatinine, it has wide variation and hence resulted in poorer performance of the abbreviated four variable MDRD2 equation in our study. The S.Cr eGFR equations were derived based on different reference methods of GFR measurement. They may hence not perform adequately satisfactorily when compared with another method of GFR estimation. In addition, DTPA clearance has not been validated against Inulin or Polyfructosan clearance in Indian population. The Gates method of DTPA scintigraphy based GFR estimation is considered inferior to the plasma sample clearance methods. The variation in the clearance measure due to technical differences adds to the accumulating errors in GFR estimation by prediction equations. This may be the reason for the relatively uniform poor precision among both the S.Cr and the CysC based equations. In addition, the validity of the Du Bois formula for assessment of body surface area in the Indian population has not been proven. Errors in this formula will add up to the variation in eGFR since all the GFR values were standardized to a BSA of 1.73Sq.m for comparability. This may be another contributor to the large S.D of bias (Precision) observed in both the CysC and S.Cr based equations.

CysC based formulae had minimal or negative bias, better accuracy and better agreement with the mGFR. The several advantages of CysC over S.Cr have already been listed in the review of literature section. CysC is not secreted in the renal tubules, thereby

minimizing errors due to secretion as observed with S.Cr. Nevertheless, Cystatin C levels may be elevated marginally with use of steroids in renal allograft recipients. To avoid this bias, we have selected patients on a stable and small dose of prednisolone (7.5 to 12.5 mg/day), unlikely to affect the CysC value significantly. Cyclosporine may reduce CysC levels thereby resulting in underestimation of GFR. The effect of Tacrolimus on CysC levels is unknown. The Larsson GFR, the Macissac GFR and the Mayo Clinic CysC GFR had better performance characteristics compared to the widely accepted Grubb GFR and should be used to estimate GFR better in the transplant recipient. The overall poor performance of all the equations prompts for further research into GFR estimation in Indian population. The future lies in deriving a specific eGFR equation for Indian population based on a gold standard technique such as Inulin or Polyfructosan clearance.

In view of these findings, several steps have been taken towards estimating GFR accurately in our setting. Firstly, CysC has been introduced as a clinical test for monthly evaluation of renal function in renal allograft recipients. Secondly, S.Cr measurement is being standardized to the IDMS traceable S.Cr as per MDRD study protocol. Thirdly, Plasma Clearance method of DTPA GFR estimation has been introduced and is currently being validated. We have also tried to acquire Polyfructosan Inutest® from Fresenius Kabi, Austria for studying GFR by gold standard method. A GFR calculator with the Larsson, Macissac and the Mayo Clinic CysC GFR equations along with the standard Creatinine based equations is being developed for ready interpretation of the CysC based GFRs in our Transplant Clinic.

SUMMARY

We studied 134 renal allograft recipients (101 males, 33 females; average age 34.0 ± 11.5years). GFR at 6 months post renal transplant was measured by 99mTc DTPA scintigraphy (mGFR). GFR was estimated (eGFR) by the various S.creatinine based equations (Cockcroft-Gault GFR, Cockcroft-Gault GFR with ideal body weight, Cockcroft-Gault GFR with lean body weight, MDRD1 GFR, Abbreviated MDRD2 GFR, Nankivell GFR and Mayo Clinic Quadratic GFR) and S.Cystatin C based eGFR equations (Le Bricon, Larsson, Grubb age adjusted, Grubb age and sex adjusted, Hoeck, Mayo Clinic CysC Transplant GFR, Macissac and Zuo Combined GFR). The mean mGFR was 52.1 ± 22.6 ml/min/1.73Sq.m. Overall, the measured GFR was different from estimated GFR. The S.Cr based equations overestimated GFR [high bias +15to20 ±25ml/min/1.73Sq.m.] and had poor correlation (0.0-0.4), poor accuracy (@30% - 35%; @50% - 55%) and poor agreement (K = 0.05-0.20; 50-60% misclassified) with mGFR. Among them, the MDRD1, MDRD2 & CG-LW GFRs had marginal correlation with mGFR (ICC= 0.25-0.35). CG-LW GFR had the least bias (+4.0 ± 22.4ml/min/1.73Sq.m; RB -32.1%) and most accuracy (@30% - 47.8%; @50% - 72.1%) among the S.Cr eGFRs. When compared to the S.Cr eGFR, all CysC eGFRs had better correlation (ICC= 0.45-0.50), lower bias (-10 to +1 ± 20ml/min/1.73Sq.m), higher accuracy (@30% - 60%; @50% - 80%) and better agreement (K = 0.25-0.45; 35-40% misclassified) with the mGFR. Among them the Larsson GFR, the Macissac GFR and the Mayo Clinic CysC TX GFR have the best performance characteristics in renal allograft recipients of Indian subcontinent.

CONLUSIONS

In renal allograft recipients of the Indian subcontinent,

1) The S.Cr based eGFR estimates poorly correlate, widely differ and rarely agree with the measured GFR (mGFR – by ^{99m}Tc-DTPA renal scintigraphy), whereas the ***CysC based eGFR estimates correlate well, differ slightly and moderately agree with mGFR***.
2) Among all eGFRs, the CysC based equations ***Larsson GFR, Macissac GFR and the Mayo Clinic CysC Tx GFR provide the best GFR estimates***
3) Among the S.Cr eGFRs, Cockcroft-Gault GFR using lean body weight provides the best GFR estimates
4) ***CysC is a better predictor of GFR than S.Cr*** at 6 months post transplantation

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PROFORMA

1. Case No.

2. Name:

3. Hospital Number:

4. Age in yrs:

5. Sex: Male – 0 / Female - 1

6. Date of Transplantation:

7. Donor – Live related – 0 / Cadaver – 1

8. Immunosuppression:

1. Pred +CsA + Aza
2. Pred + CsA + MPA
3. Pred + Tac + Aza
4. Pred + Tac + MPA
5. Others : specify:

AT 6 MONTHS:

9. Height in cm:

10. Weight in Kg:

11.S. Creat in mg/dl:

12.S.Urea in mg/dl:

13.S.Albumin in gm/dl:

14.S.Cystatin C in mg/L:

15.24 hour urine protein:

99mTc – DTPA renography

16. Renal scintigraphy performed – Yes / No

17. Compound used – Tc DTPA / DMSA / EC

18. Depth corrected? Yes / No

19. DTPA clearance in ml/min/1.73m^2:

sno	name	hospnum	age	sex	race	dotx	cada	ims	ht	wt	bsa	lbw	idbw
1	JAIRUL HAQUE	653728C	46	0	0	05-Jan-2006	0	1	168.0	57.0	1.64	47.97	64.1
2	KANU DEBNATH	678639C	24	0	0	12-Jan-2006	0	1	168.0	56.5	1.64	47.67	64.1
3	GAYATHRI	032034B	16	1	0	17-Jan-2006	1	5	154.0	53.0	1.50	39.18	50.1
4	RANGANATHAN	388879C	54	0	0	17-Jan-2006	1	1	173.0	60.5	1.72	50.90	68.7
5	KRISHNA GHOSH	684669C	33	1	0	04-Jan-2006	0	1	163.0	62.0	1.67	44.93	56.1
6	RICHARD LALNUNPUIA	745105C	29	0	0	10-Jan-2006	0	1	170.0	63.0	1.73	51.72	65.9
7	BALMAYA DAHAL	742626C	20	1	0	11-Jan-2006	0	1	150.0	52.5	1.46	38.05	47.4
8	MOHD MASUM	749468C	19	0	0	18-Jan-2006	0	1	156.0	56.0	1.55	45.11	53.3
9	RAM G GANGULI	911128B	41	0	0	08-Feb-2006	0	1	160.0	65.0	1.68	50.38	56.9
10	TSHERING TASHI	752859C	24	0	0	22-Feb-2006	0	1	162.0	47.0	1.48	40.93	58.7
11	BIJOY PRASAD	738274C	38	0	0	23-Feb-2006	0	1	166.0	61.0	1.68	49.82	62.3
12	PRAKASH BAIDYA	750298C	38	0	0	01-Mar-2006	0	1	176.0	65.0	1.80	54.04	71.4
13	SAIFUL ISLAM	726123C	30	0	0	02-Mar-2006	0	1	165.0	58.5	1.64	48.26	61.4
14	RANGARAJULU	512177C	47	0	0	07-Mar-2006	0	1	173.0	71.0	1.84	56.54	68.7
15	CHANDRASEKAR	734235C	34	0	0	08-Mar-2006	0	1	163.0	64.0	1.69	50.67	59.6
16	RATAN KANTI KHAR	740468C	50	0	0	15-Mar-2006	0	2	169.0	65.5	1.75	52.82	65.0
17	LALAN KUMAR	624491C	54	0	0	16-Mar-2006	0	1	158.0	58.0	1.58	46.55	55.1
18	GANESH KUMAR	761506C	27	0	0	21-Mar-2006	0	1	158.0	55.0	1.55	44.99	55.1
19	SITA DEVI	765975C	45	1	0	23-Mar-2006	0	1	150.0	61.0	1.56	40.79	47.4
20	FALAN MOLLA	778036C	40	0	0	28-Mar-2006	0	1	173.0	63.0	1.75	52.33	68.7
21	PAVANI LAKSHMII	334860C	22	1	0	29-Mar-2006	0	1	164.0	66.0	1.72	46.65	56.8
22	JAYANATH KR SINGHA	728810C	41	0	0	04-Apr-2006	0	1	172.0	69.0	1.81	55.30	67.7
23	HARUNAR ROSHID	710872C	32	0	0	05-Apr-2006	0	1	165.0	56.0	1.61	46.86	61.4
24	TASHI NAMGAR	752858C	21	0	0	12-Apr-2006	0	1	166.0	55.0	1.61	46.45	62.3
25	RINCHEN DEMA	786941C	23	0	0	18-Apr-2006	0	4	155.0	45.0	1.40	38.71	52.4
26	NOHRO R.C.REV.	745107C	61	0	0	19-Apr-2006	0	1	161.0	68.0	1.72	51.97	57.8
27	JYOTIRANI PANDA	765102C	35	1	0	26-Apr-2006	0	1	152.0	57.0	1.53	40.18	48.7
28	MIR MOBARAK ALI	789866C	30	0	0	02-May-2006	0	1	156.0	56.0	1.55	45.11	53.3
29	RAJ KUMAR RAWANI	750502C	24	0	0	03-May-2006	0	1	167.0	60.0	1.67	49.48	63.2
30	SHIVA KUMAR RAI	785959C	52	0	0	09-May-2006	0	1	156.0	48.0	1.45	40.68	53.3
31	MD. ZAKERHULA	780946C	25	0	0	11-May-2006	0	1	158.0	55.0	1.55	44.99	55.1
32	TUHINA SAHA	992012A	24	1	0	16-May-2006	0	1	163.0	50.0	1.52	39.57	56.1
33	RINCHEN WANGDI	802994C	34	0	0	17-May-2006	0	2	164.0	61.0	1.66	49.39	60.5
34	LALRAM DINA	817458C	32	0	0	30-May-2006	0	2	170.0	69.0	1.80	54.81	65.9
35	RAJIB BHUYAN	808528C	23	0	0	31-May-2006	0	2	177.0	61.0	1.76	51.90	72.3
36	BABITA	809363C	34	1	0	06-Jun-2006	0	4	153.0	57.0	1.54	40.45	49.4

sno	name	hospnum	age	sex	race	dotx	cada	ims	ht	wt	bsa	lbw	idbw
37	SUSHIL KUMAR JAIN	917688	51	0	0	07-Jun-2006	0	2	170.0	69.0	1.80	54.81	65.9
38	PRANNATH PAUL	762896C	42	0	0	08-Jun-2006	0	2	155.0	52.0	1.49	42.79	52.4
39	RAHUL TRIPATI	784896C	16	0	0	14-Jun-2006	0	4	178.0	70.0	1.87	57.20	73.2
40	HARIBABU K.,	129520B	39	0	0	21-Jun-2006	0	2	178.0	124.0	2.39	74.28	73.2
41	SATISH RAJ	545944C	23	0	0	22-Jun-2006	0	4	174.0	66.0	1.80	54.18	69.6
42	SUKKUR SHEIK	780000C	35	0	0	27-Jun-2006	0	2	165.0	61.0	1.67	49.61	61.4
43	SAILESH PRADHAN	814469C	35	0	0	28-Jun-2006	0	2	167.0	73.0	1.82	55.84	63.2
44	MD. ABDUL M SARKAR	729320C	26	0	0	05-Jul-2006	0	2	162.0	50.0	1.51	42.81	58.7
45	AJAY KUMAR (CAD)	766235C	31	0	0	05-Jul-2006	1	2	172.0	58.0	1.68	49.25	67.7
46	SEKAR E. (CAD)	839209C	45	0	0	05-Jul-2006	1	2	167.0	82.5	1.92	59.51	63.2
47	NAND K SARKAR	818426C	41	0	0	11-Jul-2006	0	2	175.0	74.0	1.89	58.51	70.5
48	INDU DEVI	826599C	40	1	0	12-Jul-2006	0	2	153.0	51.0	1.47	38.13	49.4
49	PRIYAVASANTH	832437C	17	0	0	19-Jul-2006	0	2	161.0	68.0	1.72	51.97	57.8
50	SHAILENDERA N SINGH	664575C	42	0	0	26-Jul-2006	0	4	172.0	57.0	1.67	48.64	67.7
51	G RAMNARAYAN REDY	844133C	27	0	0	02-Aug-2006	0	1	172.0	68.0	1.80	54.79	67.7
52	CHIRU KUMAR DEURI	451891C	18	0	0	08-Aug-2006	0	4	170.0	49.0	1.56	43.27	65.9
53	SANDHYA PANDEY	451334C	30	1	0	17-Aug-2006	0	4	153.0	46.0	1.40	35.84	49.4
54	GAURI PRASAD	826588C	34	0	0	22-Aug-2006	0	1	163.0	55.0	1.58	45.93	59.6
55	MD. SHAJADA SELIM	853273C	44	0	0	23-Aug-2006	0	2	179.0	83.0	2.02	63.78	74.1
56	PREM SAGAR CHAPLA	643990C	52	0	0	24-Aug-2006	0	4	167.0	68.0	1.76	53.58	63.2
57	JOGESH BARLA	672933C	56	0	0	30-Aug-2006	0	4	163.0	55.0	1.58	45.93	59.6
58	R P SINGH	851278C	21	0	0	31-Aug-2006	0	2	170.0	60.0	1.69	50.06	65.9
59	KHAIDEM KULBATI	767100C	31	1	0	05-Sep-2006	0	4	161.0	65.0	1.69	45.43	54.8
60	BISWAJIT BAIG	500081C	23	0	0	12-Sep-2006	0	3	173.0	48.0	1.56	42.95	68.7
61	DAWMA	886783C	48	0	0	26-Sep-2006	0	4	169.0	75.0	1.86	57.29	65.0
62	MAZNUR RAHMAN	894131C	31	0	0	27-Sep-2006	0	4	185.0	64.0	1.85	55.08	79.5
63	KAMALA DUNGAL	830515C	33	1	0	04-Oct-2006	0	4	151.0	48.0	1.41	36.40	48.1
64	SARIKA PATWA	792569C	26	1	0	05-Oct-2006	0	4	154.0	51.0	1.47	38.34	50.1
65	PRASENJIT CHOUDRY	732071c	29	0	0	12-Oct-2006	0	3	174.0	60.0	1.72	50.78	69.6
66	NURUL ISLAM	895598C	21	0	0	18-Oct-2006	0	3	160.0	54.0	1.55	44.82	56.9
67	LALRINTLUANGI	882084C	36	1	0	24-Oct-2006	0	4	159.0	64.0	1.66	44.50	53.4
68	JOSELYN C KR	879970C	58	0	0	26-Oct-2006	0	2	161.0	51.0	1.52	43.26	57.8
69	KAPTHIANGA	833825C	56	0	0	02-Nov-2006	0	4	163.0	54.0	1.57	45.35	59.6
70	PREMA RAJAN	412195C	53	1	0	07-Nov-2006	0	4	158.0	65.0	1.66	44.50	52.7
71	EBAM ZIRDO	893555C	43	0	0	08-Nov-2006	0	4	165.0	60.0	1.66	49.07	61.4
72	LALLIANZANI	846938C	49	1	0	09-Nov-2006	0	4	152.0	54.0	1.49	39.10	48.7

sno	name	hospnum	age	sex	race	dotx	cada	ims	ht	wt	bsa	lbw	idbw
73	NIRMAL KUMAR	739666C	20	0	0	14-Nov-2006	0	2	171.0	59.0	1.69	49.66	66.8
74	LALRIMNAWMA	315714C	47	0	0	15-Nov-2006	0	2	172.0	69.5	1.82	55.55	67.7
75	JASIMUDDIN	867177C	26	0	0	21-Nov-2006	0	2	174.0	65.0	1.78	53.64	69.6
76	MD. AYUB SIKDER	880480C	42	0	0	22-Nov-2006	0	4	169.0	57.0	1.65	48.14	65.0
77	LIANZUALA	429074C	50	0	0	23-Nov-2006	0	2	178.0	64.0	1.80	53.85	73.2
78	DEBASISH PAL	768978C	20	0	0	29-Nov-2006	0	3	171.0	51.0	1.59	44.71	66.8
79	RAYMAND R PANNA	507523C	33	0	0	05-Dec-2006	0	3	165.0	65.0	1.72	51.64	61.4
80	AJAY KUMAR	925604C	22	0	0	07-Dec-2006	0	4	166.0	60.0	1.67	49.28	62.3
81	SUMAN DAS	772053C	33	0	0	12-Dec-2006	0	4	148.0	47.0	1.38	38.79	46.0
82	SANGAM LEKI	905118C	22	0	0	19-Dec-2006	0	4	162.0	49.0	1.50	42.19	58.7
83	UMESH	936347C	20	0	0	21-Dec-2006	0	4	174.0	59.0	1.71	50.18	69.6
84	RAJESH JAISWAL	801482C	34	0	0	02-Jan-2007	0	4	165.0	75.0	1.82	56.05	61.4
85	INDRANI.R.	897856B	58	1	0	05-Jan-2007	1	4	157.0	66.0	1.67	44.47	52.1
86	TAHERA BEGUM	109325A	51	1	0	05-Jan-2007	1	4	156.0	67.0	1.67	44.39	51.4
87	JEYANTHI.C.	294137C	36	1	0	16-Jan-2007	0	4	151.0	52.0	1.46	38.09	48.1
88	MOHD RAFE.M.S.	868376C	27	0	0	17-Jan-2007	0	4	174.0	60.0	1.72	50.78	69.6
89	KHAJA HUSSAIN.S.P.	505466C	23	0	0	18-Jan-2007	0	4	155.0	51.0	1.48	42.24	52.4
90	RAJESWAR SINGH	944488C	48	0	0	25-Jan-2007	0	4	170.0	62.0	1.72	51.17	65.9
91	VIOLA NILI MASSEY	327707C	34	1	0	30-Jan-2007	0	4	151.0	46.5	1.40	35.72	48.1
92	VANLALRAMHLUNI	934776C	42	1	0	31-Jan-2007	0	4	158.0	59.5	1.60	42.68	52.7
93	RAGEENA VARGHESE	904754C	17	1	0	01-Feb-2007	0	4	162.0	62.0	1.66	44.66	55.4
94	JNAN R PANIGRAHI	960631C	26	0	0	13-Feb-2007	0	4	171.0	54.5	1.63	46.95	66.8
95	SHAJI MATHEW	870996C	43	0	0	15-Feb-2007	0	4	168.0	67.5	1.77	53.59	64.1
96	SANGEY REMA	854890C	20	1	0	20-Feb-2007	0	4	150.0	42.0	1.33	33.34	47.4
97	BUDHAN KONWAR	802251C	35	0	0	21-Feb-2007	0	2	166.0	56.5	1.62	47.32	62.3
98	VENKATARAMANA.C.	948589C	30	0	0	22-Feb-2007	0	4	165.0	64.0	1.70	51.14	61.4
99	HEMRAJ MANGHATE	938462C	45	0	0	27-Feb-2007	0	4	168.0	63.0	1.72	51.30	64.1
100	KHIMAN BARAL	844870C	18	0	0	01-Mar-2007	0	4	155.0	48.0	1.44	40.52	52.4
101	ANIL KUMAR GUPTA	917947C	52	0	0	06-Mar-2007	0	4	163.0	62.0	1.67	49.68	59.6
102	LAXMI B	261315C	37	1	0	08-Mar-2007	0	4	150.0	69.0	1.64	42.51	47.4
103	HEMANT KR SINGH	954815C	31	0	0	13-Mar-2007	0	4	165.0	63.0	1.69	50.64	61.4
104	UGYENLA	968357C	41	0	0	14-Mar-2007	0	2	165.0	74.0	1.81	55.65	61.4
105	LALNUHLIRI	969968C	38	1	0	22-Mar-2007	0	4	148.0	57.0	1.50	39.04	46.1
106	DASHO T GYELSHEN	506636C	54	0	0	27-Mar-2007	0	6	168.0	67.0	1.76	53.34	64.1
107	KAVITHA KUMARI	351294C	23	1	0	28-Mar-2007	0	3	160.0	53.0	1.54	40.47	54.1
108	TSHERING WANGMO	968354C	37	1	0	29-Mar-2007	0	4	149.0	49.0	1.41	36.42	46.7

sno	name	hospnum	age	sex	race	dotx	cada	ims	ht	wt	bsa	lbw	idbw
109	FAZLUL KARIM SUMON	874371C	29	0	0	03-Apr-2007	0	4	175.0	69.0	1.84	56.00	70.5
110	CHEWANG GOMDAR	343021B	54	0	0	04-Apr-2007	0	4	152.0	56.0	1.52	44.23	49.6
111	MOU CHAKRABORTHY	780879C	27	1	0	05-Apr-2007	0	3	148.0	53.0	1.45	37.73	46.1
112	MOHD SORHAB ALI	978766C	33	0	0	10-Apr-2007	0	4	168.0	62.0	1.70	50.77	64.1
113	NAND KISHORE MURMU	329734C	22	0	0	11-Apr-2007	0	4	169.0	55.5	1.63	47.25	65.0
114	WANGDUP SHERPA	826141C	19	0	0	12-Apr-2007	0	4	164.0	54.0	1.58	45.52	60.5
115	SUBRAMANI.P	994363C	33	0	0	17-Apr-2007	0	4	165.0	70.0	1.77	53.96	61.4
116	SAYAN DUTTA	830544C	17	0	0	18-Apr-2007	0	4	150.0	44.5	1.36	37.68	47.8
117	ANANDA C NAYAK	647140B	48	0	0	19-Apr-2007	0	4	173.0	48.5	1.57	43.29	68.7
118	KISHORE GURUNG	931465C	48	0	0	24-Apr-2007	0	4	154.0	60.0	1.58	46.57	51.4
119	CHANDAN RAJAK	973475C	21	0	0	26-Apr-2007	0	4	155.0	49.0	1.45	41.11	52.4
120	KAUSHIK ROY	985466C	29	0	0	01-May-2007	0	3	162.0	64.5	1.69	50.66	58.7
121	ASMA KALEEL	276802C	21	1	0	03-May-2007	0	4	156.0	60.0	1.59	42.31	51.4
122	JATINDRA MOHAPATRA	987146C	36	0	0	10-May-2007	0	4	180.0	67.0	1.85	55.97	75.0
123	HELAL AHMED	964470C	26	0	0	15-May-2007	0	4	171.0	54.0	1.63	46.64	66.8
124	MD HABILUDDIN	789397C	30	0	0	16-May-2007	0	2	153.0	47.5	1.42	39.91	50.5
125	CHANDRAKANTA D	993435C	44	1	0	23-May-2007	0	4	161.0	64.0	1.67	45.09	54.8
126	DIPA DUTTA	984100C	39	1	0	24-May-2007	0	3	150.0	47.5	1.40	35.98	47.4
127	VANLAL RATLUNGA	005572D	35	0	0	05-Jun-2007	0	4	165.0	58.0	1.63	47.98	61.4
128	AMRITA GHOI	709096B	16	1	0	07-Jun-2007	0	6	148.0	43.5	1.34	33.76	46.1
129	MD. ABDUR RAQUIB	418138C	41	0	0	13-Jun-2007	0	4	167.0	56.0	1.62	47.21	63.2
130	TARUN KUMAR	890225C	25	0	0	14-Jun-2007	0	4	166.0	55.0	1.61	46.45	62.3
131	LABONI PATI	937614C	15	1	0	20-Jun-2007	0	3	153.0	46.5	1.41	36.08	49.4
132	NAGAENDRA KUMAR	660957C	22	0	0	26-Jun-2007	0	4	183.0	67.0	1.87	56.54	77.7
133	DHAN BDUR. MO	029203D	40	0	0	27-Jun-2007	0	4	163.0	66.0	1.71	51.61	59.6
134	GOSTHA GOPAL	022399D	25	0	0	28-Jun-2007	0	4	174.0	69.0	1.83	55.77	69.6

sno	name	creat	urea	alb	cysc	dtpa	pruria
1	JAIRUL HAQUE	2.3	64	4.2	3.91	18.00	321
2	KANU DEBNATH	1.6	47	4.3	2.17	40.00	80
3	GAYATHRI	1.3	45	4.6	2.07	75.00	266
4	RANGANATHAN	1.3	45	4.2	1.25	32.40	746
5	KRISHNA GHOSH	1.1	32	4.1	1.20	79.60	116
6	RICHARD LALNUNPUIA	1.9	38	4.7	2.13	57.40	195
7	BALMAYA DAHAL	1.1	17	4.1	1.42	80.20	150
8	MOHD MASUM	1.1	15	4.3	1.20	68.00	200
9	RAM G GANGULI	1.5	37	3.9	1.82	33.90	146
10	TSHERING TASHI	0.9	27	4.2	1.23	103.00	109
11	BIJOY PRASAD	1.8	58	4.0	2.63	19.50	496
12	PRAKASH BAIDYA	1.4	30	3.9	2.00	46.00	113
13	SAIFUL ISLAM	1.3	29	4.4	1.61	66.50	151
14	RANGARAJULU	1.4	44	4.3	1.99	30.00	233
15	CHANDRASEKAR	1.7	44	4.0	2.67	32.00	166
16	RATAN KANTI KHAR	1.1	24	3.8	1.50	29.00	543
17	LALAN KUMAR	1.7	97	3.0	3.24	35.00	81
18	GANESH KUMAR	1.2	39	4.4	1.71	75.00	157
19	SITA DEVI	1.5	42	3.8	2.25	22.00	63
20	FALAN MOLLA	1.8	62	3.2	1.51	33.00	166
21	PAVANI LAKSHMII	1.0	26	4.2	1.12	56.70	52
22	JAYANATH KR SINGHA	1.0	31	3.9	1.88	74.00	99
23	HARUNAR ROSHID	1.3	22	4.2	1.46	48.00	110
24	TASHI NAMGAR	1.1	24	4.4	1.35	55.00	89
25	RINCHEN DEMA	0.8	15	4.3	1.12	112.00	64
26	NOHRO R.C.REV.	1.0	37	3.7	1.40	47.00	53
27	JYOTIRANI PANDA	1.2	44	3.6	2.31	106.00	86
28	MIR MOBARAK ALI	1.1	25	4.2	1.44	38.00	157
29	RAJ KUMAR RAWANI	1.5	39	4.0	2.31	44.00	112
30	SHIVA KUMAR RAI	1.3	28	4.1	1.93	37.00	415
31	MD. ZAKERHULA	1.4	38	4.0	2.14	49.00	335
32	TUHINA SAHA	1.1	37	4.3	1.67	84.00	104
33	RINCHEN WANGDI	1.2	42	4.2	1.53	39.20	81
34	LALRAM DINA	1.8	45	4.4	2.63	27.00	261
35	RAJIB BHUYAN	1.3	38	4.1	1.85	55.00	92
36	BABITA	1.1	34	4.0	1.48	72.00	208

sno	name	creat	urea	alb	cysc	dtpa	pruria
37	SUSHIL KUMAR JAIN	3.2	73	3.9	5.55	14.00	1,150
38	PRANNATH PAUL	1.1	29	4.3	1.69	49.00	155
39	RAHUL TRIPATI	1.3	27	4.1	1.62	36.00	228
40	HARIBABU K.,	1.2	34	3.9	2.37	28.00	107
41	SATISH RAJ	1.8	42	4.2	2.54	42.00	313
42	SUKKUR SHEIK	1.5	62	4.0	1.99	37.00	1,600
43	SAILESH PRADHAN	1.7	33	4.4	2.06	31.00	105
44	MD. ABDUL M SARKAR	1.0	24	4.3	1.15	80.00	126
45	AJAY KUMAR (CAD)	1.1	28	4.1	2.11	45.00	162
46	SEKAR E. (CAD)	1.3	35	4.5	2.06	11.00	382
47	NAND K SARKAR	1.4	27	4.5	1.81	34.60	495
48	INDU DEVI	0.9	27	4.2	1.29	80.00	97
49	PRIYAVASANTH	1.4	21	4.2	2.30	23.00	120
50	SHAILENDERA N SINGH	1.1	28	4.4	1.39	65.00	262
51	G RAMNARAYAN REDY	1.4	35	4.5	1.95	33.50	213
52	CHIRU KUMAR DEURI	1.3	43	4.5	2.00	84.00	2,600
53	SANDHYA PANDEY	1.1	25	4.4	1.13	63.00	58
54	GAURI PRASAD	0.8	24	4.6	1.04	76.60	108
55	MD. SHAJADA SELIM	1.0	19	4.5	1.28	29.00	140
56	PREM SAGAR CHAPLA	1.3	43	4.3	2.60	37.00	237
57	JOGESH BARLA	0.9	33	4.4	1.30	58.00	109
58	R P SINGH	1.5	32	4.2	1.69	65.00	75
59	KHAIDEM KULBATI	0.9	27	4.3	1.23	46.00	83
60	BISWAJIT BAIG	1.4	33	4.4	2.23	41.00	124
61	DAWMA	1.4	29	4.2	1.70	33.00	530
62	MAZNUR RAHMAN	1.5	24	4.5	1.86	32.00	136
63	KAMALA DUNGAL	1.4	26	4.0	1.64	53.00	106
64	SARIKA PATWA	1.0	26	4.3	1.20	97.00	148
65	PRASENJIT CHOUDRY	1.1	26	3.9	1.31	68.00	104
66	NURUL ISLAM	1.1	37	4.0	1.42	100.00	134
67	LALRINTLUANGI	0.9	25	4.4	1.35	75.00	58
68	JOSELYN C KR	1.5	53	3.3	1.95	83.40	143
69	KAPTHIANGA	1.3	32	4.1	1.45	57.00	87
70	PREMA RAJAN	1.1	31	3.9	1.31	53.00	60
71	EBAM ZIRDO	1.4	44	4.6	1.36	55.00	69
72	LALLIANZANI	0.9	28	3.7	1.20	113.00	49

sno	name	creat	urea	alb	cysc	dtpa	pruria
73	NIRMAL KUMAR	1.8	57	4.0	2.68	20.00	184
74	LALRIMNAWMA	1.0	24	4.3	1.34	54.00	69
75	JASIMUDDIN	1.4	31	4.4	1.55	59.00	220
76	MD. AYUB SIKDER	1.1	34	4.3	1.45	46.00	68
77	LIANZUALA	1.3	27	4.3	1.58	43.00	392
78	DEBASISH PAL	1.2	34	4.6	1.23	81.00	597
79	RAYMAND R PANNA	1.2	25	4.3	1.82	49.00	258
80	AJAY KUMAR	1.3	24	4.8	1.60	34.00	382
81	SUMAN DAS	1.1	25	4.4	1.31	82.00	171
82	SANGAM LEKI	1.0	25	4.9	1.50	43.00	213
83	UMESH	1.2	20	5.0	1.44	69.00	89
84	RAJESH JAISWAL	1.8	40	4.5	2.47	21.00	155
85	INDRANI.R.	1.2	40	3.5	1.52	24.00	72
86	TAHERA BEGUM	1.0	30	4.0	1.47	63.00	234
87	JEYANTHI.C.	0.9	23	3.8	1.46	41.00	90
88	MOHD RAFE.M.S.	1.4	38	4.5	1.91	32.00	481
89	KHAJA HUSSAIN.S.P.	1.2	29	3.3	1.66	20.00	145
90	RAJESWAR SINGH	0.9	29	4.0	1.29	44.00	180
91	VIOLA NILI MASSEY	0.9	18	4.1	1.17	29.00	229
92	VANLALRAMHLUNI	0.8	21	4.3	0.98	74.00	164
93	RAGEENA VARGHESE	0.9	19	4.0	0.96	71.00	160
94	JNAN R PANIGRAHI	1.1	32	4.2	1.38	57.00	164
95	SHAJI MATHEW	1.2	40	4.7	0.97	54.50	191
96	SANGEY REMA	0.7	28	4.1	1.07	69.00	48
97	BUDHAN KONWAR	1.5	34	4.2	2.23	35.00	383
98	VENKATARAMANA.C.	1.2	24	4.0	1.29	53.00	146
99	HEMRAJ MANGHATE	1.3	24	4.3	2.05	21.50	131
100	KHIMAN BARAL	1.0	24	4.6	1.20	60.90	240
101	ANIL KUMAR GUPTA	1.0	37	4.4	1.26	40.60	87
102	LAXMI B	0.8	21	4.1	1.24	37.60	187
103	HEMANT KR SINGH	1.4	35	4.8	1.60	35.00	188
104	UGYENLA	1.3	21	4.4	1.60	30.00	127
105	LALNUHLIRI	0.8	20	3.9	0.95	89.40	147
106	DASHO T GYELSHEN	1.0	33	4.7	1.06	61.20	79
107	KAVITHA KUMARI	0.8	17	4.6	1.22	60.30	178
108	TSHERING WANGMO	0.7	31	4.5	0.89	56.40	85

sno	name	creat	urea	alb	cysc	dtpa	pruria
109	FAZLUL KARIM SUMON	1.2	20	4.4	1.51	42.60	143
110	CHEWANG GOMDAR	1.5	85	4.3	2.15	17.40	375
111	MOU CHAKRABORTHY	0.9	26	4.2	1.00	77.70	113
112	MOHD SORHAB ALI	1.8	50	4.5	2.34	21.30	553
113	NAND KISHORE MURMU	2.2	110	2.6	3.75	25.00	3,100
114	WANGDUP SHERPA	1.8	39	4.6	1.70	33.00	163
115	SUBRAMANI.P	1.8	32	4.5	1.69	19.00	400
116	SAYAN DUTTA	1.1	33	4.5	1.33	59.00	93
117	ANANDA C NAYAK	0.8	15	4.1	1.20	74.00	262
118	KISHORE GURUNG	1.0	28	4.7	0.92	47.50	95
119	CHANDAN RAJAK	1.0	29	4.6	1.20	77.00	480
120	KAUSHIK ROY	1.2	34	4.6	1.30	57.00	109
121	ASMA KALEEL	0.9	37	4.3	1.27	43.30	121
122	JATINDRA MOHAPATRA	1.6	38	4.2	2.41	24.00	221
123	HELAL AHMED	1.2	35	4.7	1.44	45.00	218
124	MD HABILUDDIN	1.1	28	4.4	1.62	97.00	117
125	CHANDRAKANTA D	1.1	25	4.0	1.43	41.20	150
126	DIPA DUTTA	1.2	38	4.1	1.56	58.00	144
127	VANLAL RATLUNGA	1.3	24	4.4	1.49	49.83	297
128	AMRITA GHOI	0.8	27	4.0	1.11	72.00	150
129	MD. ABDUR RAQUIB	1.2	26	4.8	1.41	53.00	163
130	TARUN KUMAR	1.0	31	4.7	1.29	59.00	330
131	LABONI PATI	0.9	35	5.0	1.39	75.00	254
132	NAGAENDRA KUMAR	1.4	24	5.1	1.18	49.00	215
133	DHAN BDUR. MO	0.9	29	4.6	1.19	91.00	136
134	GOSTHA GOPAL	1.5	46	4.8	1.53	62.00	107